1951

Anthracnose fungi on sycamore and oak in Iowa

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NOTE TO USERS

ANTHRACNOSE FUNGI ON SYCAMORE AND OAK IN IOWA

by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Plant Pathology

Approved:

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In Charge of Major Work

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1951

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INTRODUCTION

Anthracnose of white oak and sycamore is caused by Gnomonia veneta (Speg. & Sacc.) Klebahn. The disease results in severe defoliation in early spring and fall when climatic conditions are favorable and may cause extensive twig blight and destruction of the growing points. The damage from this fungus is not fully realized for in addition to the distress of homeowners occasioned by the fallen leaves on their lawns, both terminal and radial growth may be drastically reduced. Seedling trees in the nursery row may be severely damaged or killed, and repeated attacks on larger trees have been reported to result in death (52).

The disease has been induced by inoculation of sycamore and oak with conidial isolates from these hosts, but the precise conditions relative to the development of the disease in the field and in the greenhouse are not known. More information is necessary regarding the germination of spores in vitro and on the host, and about the manner in which infection occurs naturally. The fungus overwinters in the twigs, but proof of how the mycelium becomes established has not been shown.

The relationship of conidial isolates from white oak and sycamore and their associated sexual stages is not clearly understood. A study of the sexual stages indicated only one species was involved (13), however, constant differences observed in studies of conidial isolates indicated more than one fungus is responsible for the disease (51, 63).

To clarify the relationships of these fungi extensive conidial isolations were made from infected trees, and sexual stages were examined on overwintered leaves. To study the other related problems, growth factor requirements were determined, spore germination tests were conducted, inoculation methods and the movement of these fungi in host tissue were investigated. To control the disease two spray tests were carried out; one to determine what spray date combinations are most effective, and the other to test the effectiveness of five fungicides.

REVIEW OF LITERATURE

Sycamore and Oak Anthracnose

Anthracnose on sycamore (Platanus sp.) and white oak (Quercus alba) is commonly present wherever the host is grown. The leaf blight appears soon after the young leaves start to expand in the spring and is particularly severe after periods of prolonged rains and cool temperatures. Mature leaf infection later in the season characteristically produces elongate brown streaks along the petioles and midveins. In addition to reduction in growth from this loss of foliage, sycamores frequently undergo extensive twig blight; however, twig infection on white oak, though common, is less severe. Schuldt (51) described in detail and illustrated the symptoms on white oak, sycamore and other forest trees.

The imperfect stage of the fungus causing this disease was first named Hymenula plantani by Leveille (28) in 1848. Fuckel (17) changed the name to Fusarium nervisequum, and Saccardo (49) transferred it to the genus Gloeosporium. The name Gloeosporium nervisequum (Fckl.) Sacc. was used by Ellis and Everhart in their descriptions of North American species of Gloeosporium (14).

One of the first reports of a severe anthracnose infestation was made by Southworth (54, 55) in 1889. She stated that the disease had been observed in Ohio, Kentucky, Indiana, New Jersey, Illinois, California and Iowa, and was also present in Germany, Italy and France. Platanus

racemosa, P. orientalis, P. occidentalis and Quercus were reported as hosts. The disease occurred not only on the leaves and petioles but also on the unlignified stems.

Fritz von Tavel (61) indicated that the twig blight attributed to Discula platani (Pk.) Sacc. might be only another stage of Gloeosporium nervisequum since both occur in the same vicinity and the spores are very similar. Leaves and branches of Platanus were not successfully inoculated with either Discula or Gloeosporium, so he was unable to prove the relationship of the two forms.

In 1890 the disease was described on oak and sycamore by Halsted (22, 23). He identified the causal agent as Gloeosporium nervisequum, and also reported the disease on a red maple tree. The fungus was also reported on oak and sycamore by Stoneman (56) and the same general symptoms were described. She experienced difficulty in obtaining pure cultures. The spores were described as being ovate oblong or oblong measuring 10-12 x 4.5-5.0 μ.

The first worker to demonstrate that Gloeosporium is an imperfect stage of Gnomonia was Klebahn in 1905 (26). The perfect stage was found in late winter and early spring on old diseased sycamore leaves that had been under the trees during the winter. Klebahn identified this perfect stage as Laestadia veneta Sacc. & Speg. but transferred it to the genus Gnomonia with the binomial name G. veneta (Sacc. & Speg.) Klebahn. He also concluded that the numerous conidial forms described as separate species depending on where they occurred on the host were actually stages of Gloeosporium. He placed in synonymy Gloeosporium nervisequum whose

conidia are borne in acervuli on short sporophores under the cuticle, Gloeosporium platani (Mont.) Oud., with conidia borne in acervuli on long sporophores under the epidermis, Myxosporium valsoideum (Sacc.) All. and Discula platani (Peck) Sacc. conidia borne in pustules on twigs and Sporonema platani Baumler and Fusicoccum veronense C. Massalongo conidia borne in cleistocarpus pycnidia on old leaves on the ground. Klebahn obtained only limited infection from pure cultures applied on the leaves.

Edgerton (13) in 1908 confirmed Klebahn's findings on sycamore and also reported the fungus became actively saprophytic and continued to grow and produce viable conidia and perithecia on dead leaves. Since the conidia and ascospores were held together by a mucilaginous substance, he assumed they were not wind borne and probably ineffectual in causing the spring leaf blight. The inoculum produced on young twigs was considered most important in initiating infection in the spring.

White oak and sycamore anthracnose were usually considered to be caused by the same organism, however, isolates from oak had not been associated with a sexual stage. Edgerton found a perithecial stage on dead oak leaves and concluded it was the same species as that found on sycamore. The ascospores, asci and perithecial characteristics of the fungus from oak and sycamore were similar except on the oak the perithecial necks were longer. He stated the conidia from oak were somewhat larger than those on sycamore but after the fungus had grown in pure culture, the conidia were nearly the same size as those from sycamore. He attempted to inoculate sycamore leaves on cut branches and kept fresh by standing in water in the laboratory. None of the leaves became infected.

The fungus was found on the petioles of sycamore by Anderson (2) who suggested that mycelium progressed internally to the twigs.

Westerdyk and Van Luijk (63) believed that Gloeosporium on oak and sycamore differ enough to be separate species. Conidia from the leaves of Quercus pedunculata, Q. rubra, Q. coccinia and Platanus occidentalis were 12.3, 13.2, 12.6 and 9.7 μ in length, respectively. The spores from leaves were more uniform in size than from pure culture, but the same relative differences held. They proposed that the Gloeosporium from oak be referred to as Gloeosporium quercinum West.

A heavy infestation of sycamore blight in Italy was observed by Sempio (52). He noticed that the fungus spread from twigs to branches where it caused large cankers and postulated the formation of a toxin because of the pronounced local hypertrophy of these tissues. A brown lesion developed at the nodes of the twigs and extended into the cambium. The swollen tissue on the branches contained distorted vascular tissue. The cells contained heavy inclusions of a reddish brown substance. Mycelium was scarce in living tissue but abundant in dead sections. He believed that inoculum from twigs was responsible for primary infection even though ascospores probably could induce infection. Pruning out the diseased twigs and branches was recommended for control. Infection was not induced consistently on seedlings exposed to conidia from pure cultures. Acervuli did develop, however, on two leaves one month after exposure to the fungus.

For years the standard recommendation for control of sycamore anthracnose has been three applications of Bordeaux mixture or sulfur.

Recently some of the newer organic fungicides have been used. Carter (10) tested 22 organic fungicides and found phenylmercuri-triethanol ammonium lactate most effective in preventing the disease, with only 3 percent of the leaves on sprayed trees and 60 percent of those on unsprayed trees being infected. In 1948 Dimond and Potts (12) applied yellow cuprous oxide, ferric dimethyldithiocarbamate, copper naphthenate and phenylmercuri-triethanol ammonium lactate with a mist blower. Phenylmercuri-triethanol ammonium lactate gave the best control. Phytotoxicity was rated in decreasing order as follows: copper naphthenate, yellow cuprous oxide, phenylmercuri-triethanol ammonium lactate and ferric dimethyldithiocarbamate.

Growth Factor Requirements of Fungi

The growth of many fungi is determined by the presence or absence of growth factors in the culture medium (47). Very often these growth factors are members of the vitamin B complex, their components or derivatives (44, 45). A knowledge of these growth factors is of importance not only for the culture of specific fungi but as an aid in separating closely related groups or to add to our understanding of the basic factors concerned in the development of all organisms.

Fungi may display one or more vitamin deficiencies. If they are unable to synthesize any of the vitamins in question, they will not grow and are said to have a complete deficiency. This is true of Phycomyces, first shown by Schopfer in 1934 (50). Other fungi exhibit partial defi-

ciences; they will grow slowly in the absence of a particular vitamin but more rapidly if it is present. Complete and partial deficiencies may exist for one vitamin or several vitamins and are said to be absolute, if not changed, or conditioned, if changed by environmental conditions. The relation of Phycomyces to thiamin appears to be absolute, while the relation of thiamin to Pythium butleri is conditioned because the thiamin deficiency is removed by reducing the concentration of the mineral salts (46).

The ability of fungi to synthesize a particular vitamin or its part is variable with each organism. Phytophthora cinnamoni must be supplied with the complete thiamin molecule (40), while Mucor ramannianus can make the pyrimidine half of the thiamin molecule but not the thiazole portion (38). The thiazole part can be made by Sclerotium rolfsii but not the pyrimidine part (43); Phycomyces is unable to synthesize either part but can put them together (42, 53).

Few of the dozen or more chemically pure vitamins now available have been found to be growth substances for fungi. Thiamin or its components is the most common deficiency for fungi (48, 41, 29); biotin has been frequently reported (24, 25, 4) and pyridoxine (48) and inositol (16) have been reported for a few fungi. Beadle and Tatum (5) have reported mutant strains of Neurospora which are deficient in pyridoxine and para-amino benzoic acid. Deficiences for most of the other vitamins have not been reported. Many fungi display a complete or partial deficiency for one or more vitamins (47).

Some fungi are dependent on vitamins for sporulation as well as growth. An isolate of Ceratostomella fimbriata, completely deficient

for thiamin, produced perithecia only when the ratio of thiamin to the amount of nutrients in the medium was relatively high. Sexual reproduction required the presence of more thiamin than was required for mycelial growth (3). Melanospora destruens produced no perithecia on a mineral-dextrose medium plus 1.5 percent agar. The addition of inositol, biotin, thiamin, biotin and inositol, or thiamin and inositol did not stimulate perithecial formation, but the addition of biotin and thiamin, or biotin and pyrimidine stimulated the formation of perithecia (24, 25).

Lilly and Barnett (30) studied the influence of concentrations of basal nutrients, thiamin and biotin on the growth and formation of perithecia and ascospores by Chaetomium convolutum. The amount of vegetative growth was controlled by the amount of nutrients present and the concentration of thiamin and biotin. Perithecia were formed only after vegetative growth reached a maximum. For any given concentration of nutrients, a corresponding concentration of thiamin and biotin was required before mature perithecia developed. Low concentrations of these vitamins allowed perithecia to form but mature ascospores did not develop. Sordaria fimicola, deficient for biotin, produced perithecia only after the pH of the medium had risen above six, but the effect of pH alone was not sufficient to insure sexual reproduction. An adequate amount of biotin was necessary for perithecial formation, since biotin concentration influenced the number of perithecia formed (4). With many other fungi sporulation as well as growth was influenced by vitamins (47).

Fungi have been found useful in vitamin bioassays (27). The fungi used must have a complete deficiency for a given vitamin and must respond at very low concentrations.

The literature indicates that a great number of fungi require growth substances, and future research will no doubt add to the list of organisms for which these substances need to be supplied in culture. The response of fungi to growth factor requirements has been used to distinguish closely related organisms.

Factors Which Affect Spore Germination

The physiology of spore germination has long been of interest to mycologists and plant pathologists (20). Germination differs from mycelial growth in that spores have their own stored food reserves and are able to germinate in water or other simple media. The germination process is characterized by a change from low to high metabolic activity (15, 18, 19) and by swelling and germ tube production (37).

Water is one of the most important factors for spore germination and for many fungi is the only material necessary to start the process (6). The first visible evidence of germination is the swelling of the spore as water is taken up; some spores becoming twice their original size (36, 37). Mandels and Norton (35) point out that the respiration rate of Myrothecium verrucaria spores increases very rapidly after water enters the cell and before the germ tube appears. In the presence of nutrients this fungus may increase its dry weight during germination, indicating synthetic activity may also accompany germination.

The water requirements of fungi differ, some require liquid water and for others water vapor is sufficient. Clayton (11) showed that the mean percentage germination of uredospores of Puccinia coronata, P. graminis

tritici (race 56), and of P. graminis avenae (race 2) on glass was high in water, lower at a relative humidity of 100 percent, considerably lower at 99.0 and practically zero at 98 percent. He also demonstrated for the first time that ascospores and conidia of Venturia inaequalis could germinate on dry glass at relative humidities of 99 to 100 percent. Brodie and Neufeld (7) reported germination of conidia from Erysiphe polygoni and Erysiphe graminis through a range of relative humidity from approximately zero to 100 percent. Tompkins (58, 59) stated that only the vapor phase of water is active, since some spores germinated as well, or better, in a saturated atmosphere as when floating in water and hardly germinated at all when submerged.

It has long been recognized that water alone is insufficient for the germination of many fungi, and that germination is increased by the addition of natural products to the medium (8, 9). Spores of some phytopathogenic fungi are stimulated by material from leaves or extracts from other plant parts such as apple juice, orange juice, tomato juice, potato and others (33, 9). The effects of such materials are usually attributed to growth-promoting substances, but none of these effects has been demonstrated to be due to known vitamins (35, 36).

Carbohydrates, such as glucose and sucrose, have stimulated germination most frequently (21, 31, 32), and nitrogen sources have also been implicated (32, 37). Studies on spores of Sclerotinia fructicola by Lin (31, 32), showed that spores collected by brushing a culture or flooding it with water would germinate about 85 percent, but failed to germinated when thoroughly washed and centrifuged. When carbohydrates

such as dextrose or sucrose were added to the washed and centrifuged spores, good germination was recorded indicating that sufficient carbohydrate for good germination was not stored in the spores.

Most fungi tend to give maximum germination in the acid range and only rarely is germination favored by an alkaline reaction. An optimum of about pH 3 has been reported for many fungi (57, 62), but others germinate best between pH 6 and 7.0 (34, 62). Webb (62) points out that differences in temperature do not affect the pH optimum, however, the composition of the medium may have an effect.

METHODS AND MATERIALS

Source of Cultures

Most of the cultures used in these investigations were obtained from naturally infected leaves and twigs of sycamore and oak; however, since anthracnose also occurs on many of our common shade and forest trees additional cultures were obtained from elm, walnut, ash and hickory. Pieces of infected blades, midviens and petioles of leaves or twigs were surface disinfected by immersion in sodium hypochlorite solution (20 percent dilution of Clorox) for one minute and rinsed twice in sterile distilled water. Woody tissue was placed in 95 percent ethyl alcohol for 30 seconds, then flamed. After surface disinfection, the diseased tissue was transferred to a sterile glass slide. Small sections cut with a flamed scalpel were then planted on potato-dextrose or yeast extract agar and incubated at room temperature.

Single spore cultures were obtained by pouring 2 ml. of a dilute spore suspension on a 2 percent water agar plate. After the agar had absorbed the excess water a spore was located under the low power of the microscope and transferred by use of a small "biscuit cutter" to a plate containing potato-dextrose agar. After the spore had germinated and growth occurred, transfers were made to a potato-dextrose agar slant. In making single ascospore isolates, greater care was necessary in preparation of the

initial dilute spore suspension to avoid excessive bacterial contamination. The perithecia were first removed from the leaf tissue, washed several times in sterile water, and then broken open to release the spores. The spores were then handled as described above.

Two methods were used to encourage the production of the ascogenous stage on infected oak and sycamore leaves collected in June and July. In the first method infected leaves were put between layers of moist sphagnum moss in a greenhouse flat, covered with wire screen and incubated at 33°F. At monthly intervals the flats were checked and enough water added to keep the leaves moist. The second method employed sterile Petri plates containing 20 ml. of 2 percent water agar. Pieces of infected leaf tissue, surface disinfected and washed in sterile water, were planted on these plates and incubated at 5° and 10°C. The leaves were checked at monthly intervals for perithecial formation.

Spores from young cultures or perithecia mounted in lactophenol were measured under oil immersion (970X) with an ocular micrometer.

From 300 isolations made from leaves and twigs infected with anthracnose about 75 representative cultures were selected and maintained on potato-dextrose agar slants. These isolates produced only the conidial stage and in this thesis are referred to as the genus Gloeosporium of the Fungi Imperfecti. Due to rather distinct morphological and physiological characteristics, the conidial isolates are placed in two groups. Isolates of the "oak group" have been obtained from white oak, red oak, American elm and black walnut but have never been obtained from sycamore. Isolates of the "sycamore group" have been obtained from sycamore, white oak and bur oak.

The history of some of the representative conidial isolates used in many of the detailed studies are as follows:

Oak group

Isolate B-3-3 was obtained from the midrib and petiole of a white oak leaf which showed no anthracnose symptoms. The same fungus was isolated from apparently healthy new growth and one year old wood. The tree showed anthracnose symptoms on other leaves.

Isolate AP-103 was obtained from red oak. This isolate appeared in a plate in which a routine isolation had been made for the oak wilt fungus. The tree showed no anthracnose symptoms. Three other similar isolates of Gloeosporium were obtained from red oak trees showing no symptoms.

Sycamore group

Isolate G-2 was obtained from an apparently healthy midrib of a sycamore leaf. Similar isolates were obtained from infected twigs and leaves.

Isolate MO-1-1 was obtained from the midrib and petiole of an infected white oak leaf collected near Holland, Michigan. Leaves on the tree were 95 percent destroyed.

Growth Factor Requirements

In measuring the effect of growth factors on these isolates a basal medium modified after the medium of Robbins and Kavanagh (43) was prepared according to the following formula:

Constituent	Conc. per liter
KNO_3	1.0 gm.
NH_4NO_3	1.0 gm.
KH_2PO_4	0.5 gm.
$MgSO_4$	0.25 gm.
Dextrose	20.0 gm.
Trace elements:	
$FeSO_4$	0.2 mg.
$ZnCl_2$	0.5 mg.
$CuSO_4$	0.14 mg.
$(NH_4)_6Mo_7O_{24}\cdot H_2O$	0.03 mg.
$MnSO_4\cdot 4H_2O$	0.14 mg.

As amendments, various combinations of the following supplements were added to this basal medium at the concentrations indicated:

Constituent	Conc. per liter
Thiamin	0.2 mg.
Para-aminobenzoic acid	0.6 mg.
Biotin	0.5 micrograms
Calcium pantothenate	0.48 mg.
Choline chloride	4.0 mg.

Constituent	Conc. per liter
Inositol	40.0 mg.
Folic acid	1.0 mg.
Nicotinic acid	1.0 mg.
Pyridoxine hydrocholide	0.1 mg.
Riboflavin	0.3 mg.

The basal medium was further amended for some tests by the addition of vitamin-free casein hydrolysate, 2 gm. (casein equivalent) per liter, vitamin B-12, 1.5 micrograms per liter and yeast extract (Bacto), 4 gm. per liter. These amendments are not included when reference is made to supplements.

Representative isolates from both the oak and sycamore groups were used in these tests and also single ascospore isolates from white oak and sycamore. The tests were replicated at least four times using 125 or 250 ml. Erlenmeyer flasks with 50 ml. of medium per flask. The pH of the medium was adjusted and after autoclaving it was about pH 5.3. Each flask was seeded with a uniform spore suspension made with sterile water and incubated at 20 - 25°C. After an appropriate period of growth, the mycelial mat was filtered under suction on previously weighed filter paper, then dried and weighed to the nearest milligram. Blanks were processed with each test to accurately equilibrate the paper to the same standard of dryness. Precision of plus or minus 1 mg. was usually attained. To increase the growth rate, a rotary swirl shaker was used in some tests.

Growth factor studies made with agar plates employed the same basal medium with the addition of 20 gms. of agar per liter. Combinations of

supplements were added at the concentrations previously indicated. Quadruplicate plates were centrally inoculated with a small droplet of spore suspension with a sterile pipette. Growth was recorded as increase in colony diameter.

To study the effect of thiamin on sporulation the above technique was employed with the following additions to the basal medium: (1) all supplements except thiamin, (2) thiamin, biotin and inositol, (3) yeast and (4) no additions. To determine the number of spores over a uniform area of plate surface where growth occurred three circles selected at random were cut in the agar surface with a No. 3 cork borer (diameter 7 mm.) from each of the four plates in each treatment. The agar circles supporting the spores and mycelium were removed to 10 ml. of water. The spores were dislodged by mascerating the agar with a stirring rod and shaking vigorously. The number of spores per ml. was determined with a Levy counting chamber.

Germination and Respiration of Spores

The effect of oleic acid, linoleic acid, dextrose and sodium oleate on spore germination was determined by a glass slide germination test modified from that recommended by the American Phytopathological Society's committee on standardization of fungicidal tests (1). Six uniform droplets of spore suspension were put on each slide with a micropipette. The surface area of the droplets was controlled by small vasoline rings stamped on the slides with a No. 00 rubber stopper bored with a No. 2 cork borer.

After incubation for 24 hours, 100 spores were counted in each of the six droplets.

To determine the optimum hydrogen-ion concentration for germination, spores from isolate B-3-3 from white oak were suspended on glass slides in droplets of citric acid-phosphate buffered solutions from pH 3 to 8 and counted as described above.

The drying rate of droplets on glass slides was controlled in sealed chambers by varying the relative humidity with concentrations of NaCl in water as follows:

Relative humidity	Grams NaCl per 100 gm. of water[1]
100	0
99	1.50
98	3.25
97	5.00
96	6.60
95	8.25

Respiration rates of spores from Gloeosporium isolate B-3-3 were determined by measuring O_2 uptake in the Warburg constant volume respirometer (60). This isolate grown on yeast agar plates sporulated abundantly with the surface of the plate almost devoid of mycelium. The spores were removed by flooding the plate with sterile distilled water

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[1] This information was supplied by Dr. George Semeniuk, Iowa State College, Ames, Iowa.

and agitating the surface with a rubber policeman. The spores were then filtered through cheesecloth to remove bits of agar or mycelial fragments and washed twice by centrifugation. The centrifuged spores were diluted with six volumes of water and 1 ml. was added to each respirometer flask. Other additions were 0.3 ml. of 0.2 molar phosphate buffer pH 6.0; 0.2 ml. of 20 percent KOH (in the center well) and 1.5 ml. of test materials to make a total volume of 3 ml. The temperature was held at 30°C. The effect of the following materials on O_2 uptake was studied; oleic acid, linoleic acid, sodium oleate and dextrose.

Inoculation

Two methods were used to determine the virulence of cultures for one year old seedling trees grown in the greenhouse. Spores were atomized on leaf surfaces or spore suspensions were injected into young actively growing shoots. To encourage germination when spores were atomized on leaf surfaces, sodium oleate solutions adjusted to pH 7.5, or oleic acid at the rate of 0.25 or 0.50 percent was incorporated into the spore suspensions. After exposure to the spore suspension the plants were placed in an incubation chamber maintained at 60 to 80°F. and 100 percent relative humidity. Trees sprayed with a spore suspension were kept in the chamber until symptom expression occurred. Trees inoculated by use of a hypodermic syringe were kept in the moist chamber from 24 to 48 hours and then removed.

Free hand sections of fresh material were cut from apparently healthy and diseased tissue and put into lactophenol to which had been added 0.5 percent acid fuchsin and 0.5 percent cotton blue (39). This preparation

was heated until bubbles appeared and then allowed to cool. The sections were washed with lactophenol and mounted on slides for study.

Control

In 1950, sprays were applied to 187 trees 25 to 50 feet high on Polk Boulevard and to 155 trees 50 to 80 feet high on Thompson Avenue in Des Moines, Iowa. The chemicals were applied with hydraulic equipment, mounted on a truck, with a 400 gallon capacity and 600 pounds pressure at the spray gun. The amount of infection was determined by counting the number of infected leaves that could be seen from the ground. Infected leaves that were still green but had fallen were added to the total number of infected leaves per tree. The central three trees of the five in each treatment were counted on Polk Boulevard and the central two of four on Thompson Avenue. This was done to eliminate as much as possible the effect of spray drift between treatments.

On Polk Boulevard blocks of five trees per treatment in six replications were sprayed with the following materials at the rates indicated: tribasic copper sulfate (Tennessee Tribasic Copper Sulphate), 4 pounds per 100 gallons; Bordeaux mixture, 4-4-50; ferric dimethyldithiocarbamate (Fermate), 1-1/2 pounds per 100 gallons; 2,3-dichloro-1,4-naphthaquinone (Phygon), 3/4 pound per 100 gallons; and phenylmercuri-triethanol ammonium lactate (Puratized Agricultural Spray), 1 pint per 100 gallons. Spray applications were made on April 7, May 13, May 29 and June 16; records were taken on June 3, 8 and 20.

On Thompson Avenue a test of nine treatments of four trees per treatment in 4 replications was designed to determine what combination of sprays corresponding to progressive stages of development of the new leaves is necessary to control sycamore anthracnose with phenylmercuri-triethanol ammonium lactate. Combinations of the following 4 sprays were used: (1) April 7, dormant spray; (2) May 13, broken bud spray; (3) May 27, young leaf spray; and (4) June 16, mature leaf spray. Of the possible spray date combinations the following seven were chosen: spray No. 1; spray No. 2; sprays No. 1 and 2; sprays No. 2 and 4; sprays No. 2 and 3; sprays No. 1, 2 and 3; and sprays No. 1, 3 and 4. This experiment was carried out in 1949 and one treatment which had been sprayed three times in 1949 was selected to measure carry-over value and designated as the carry-over check in 1950. The check was unsprayed in 1949 and 1950. Records were taken on June 3 and 20.

EXPERIMENTAL RESULTS

Comparison of Anthracnose Fungi from Oak and Sycamore

During the past three years pure cultures of Gloeosporium sp. have been isolated from anthracnose infected leaves and twigs of sycamore, oak, elm and walnut. Differences in spore size and shape, growth-temperature relationships and cultural characteristics seemed to warrant placing these isolates in two separate groups. Isolates from white oak, red oak and single isolates each from American elm and black walnut are designated as the "oak group". The isolates from American elm and black walnut are distinct from the anthracnose fungi normally associated with these hosts; Gnomonia ulmea (Schw.) Thum., black spot of elm and Gnomonia leptostyla (Fr.) Ces. & De Not., leaf spot of walnut. The "sycamore group" includes isolates from sycamore, bur oak and white oak. These groups are comparable to those outlined by the author previously (56), except that they are expanded.

In the early literature (23, 54, 56) sycamore and oak anthracnose was thought to be caused by the same organism, Gloeosporium nervisequum. Klebahn (26) found the sexual stage associated with the Gloeosporium on sycamore and called it Gnomonia veneta. Edgerton (13) varified these findings, and also found a perithecial stage on dead overwintered white oak leaves which differed only in the length of the perithecial neck. Conidia from oak were somewhat larger than those on sycamore at first,

but became nearly the same size when grown in pure culture. Edgerton considered these fungi from oak and sycamore to be the same species. Westerdijk and Van Luijk (63) believed Gloeosporium on oak and sycamore differed enough on the basis of conidial size to be separate species, and proposed that the one from oak be referred to as Gloeosporium quercinum West.

To obtain additional evidence relative to the identity of these two groups of fungi, the sexual stages on oak and sycamore were studied. Since these fungi do not produce perithecia on nutrient agar, infected leaves were collected from white oak and sycamore in June and July, kept moist with sphagnum moss or on water agar plates and incubated at 3° to 10°C. Perithecial initials were seen by October and the first mature ascospores were observed in December.

The principal difference between the perithecia on the two hosts is the long beak formed on white oak leaves and the short beak on sycamore (Fig. 1). This difference in relative beak length is constant when perithecia are formed on overwintered tissue. The perithecia have the same size range on the two hosts, are separate, innate and may finally be erumpent as shown in Figure 2. The beaks may protrude from either side of the host tissue.

When asci and ascospores from the two hosts are compared, no obvious distinctions are discernible (Fig. 3 and 4). The ascospores are asymmetric in shape, have rounded ends and two cells, the smaller only 1/5 to 1/4 the length of the larger. There are eight ascospores per ascus, generally in two rows. The thickened apex of the asci have a pore which is surrounded by a ring (Fig. 4). When precise measurements are made under oil immersion

(970X), the ascospores from sycamore leaves average 1.0 micron longer and 0.1 micron narrower in width than ascospores from white oak (Table 1). The asci from sycamore are slightly larger than those from white oak (Table 1). When the "t" test is applied to measurements of asci and ascospores from the two hosts a significant difference at the 1 percent level is found between ascospore length, ascus length and width. No significant difference was found between ascospore widths. It is doubtful that these observations provide sufficient justification for separation.

Table 1. Comparison of ascospores and asci from perithecia produced on overwintered white oak and sycamore leaves.

Material	Host	Length and width of 100 ascospores and asci	
		Range (μ)	Mean (μ)
Ascospores	White oak	10.1-15.8 x 2.9-5.8	13.4 x 3.7
Ascospores	Sycamore	11.5-16.6 x 2.9-4.7	14.0 x 3.6
Asci	White oak	34.6-50.4 x 7.2-11.5	40.8 x 8.2
Asci	Sycamore	34.6-50.4 x 7.2-11.5	41.9 x 8.7

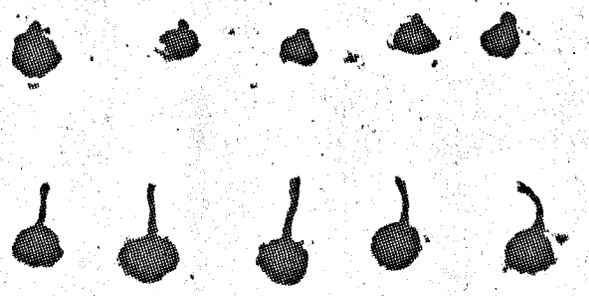
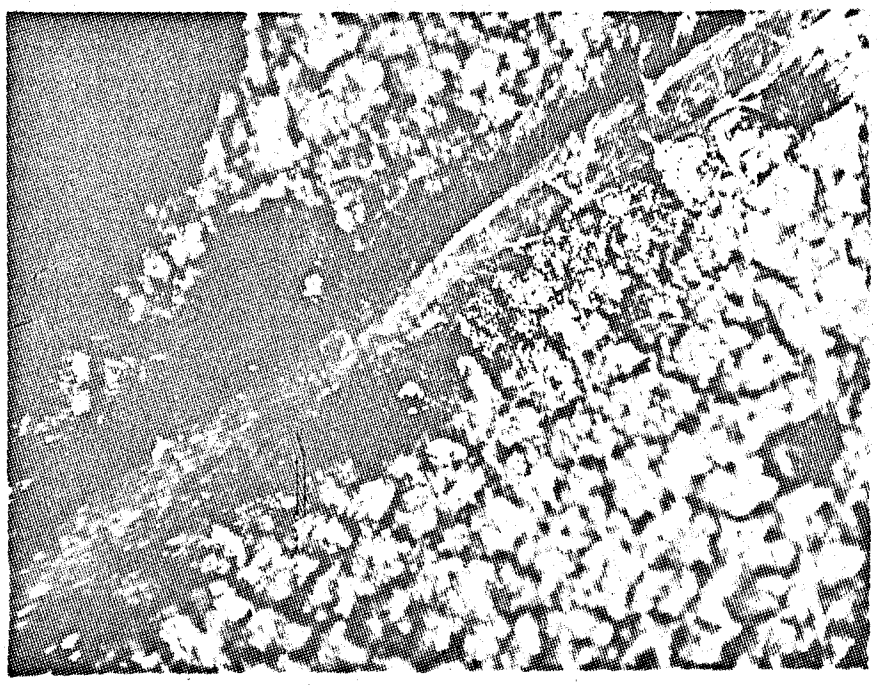
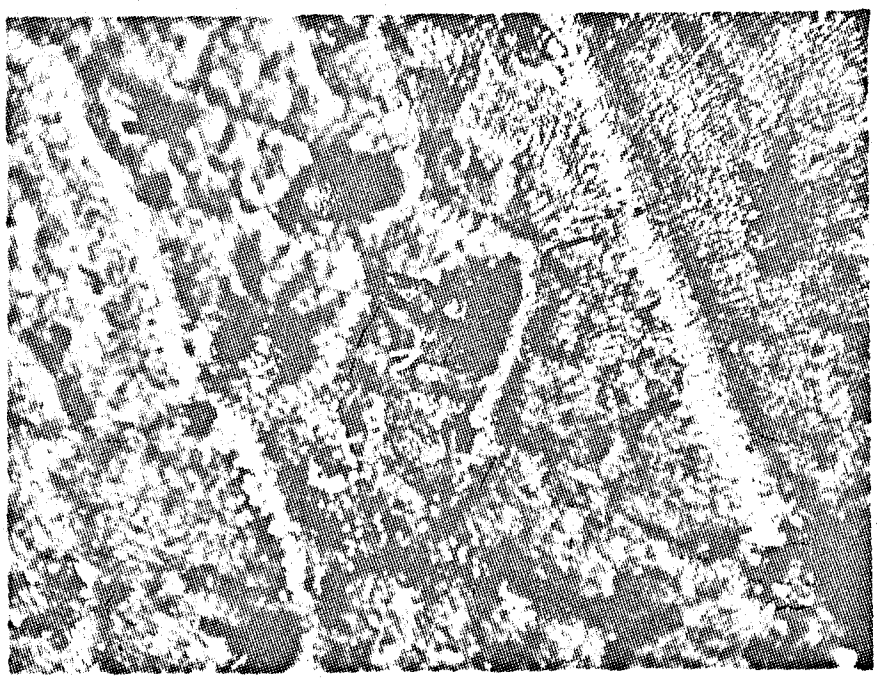


Fig. 1. Perithecia from overwintered sycamore leaves above, and white oak leaves below.

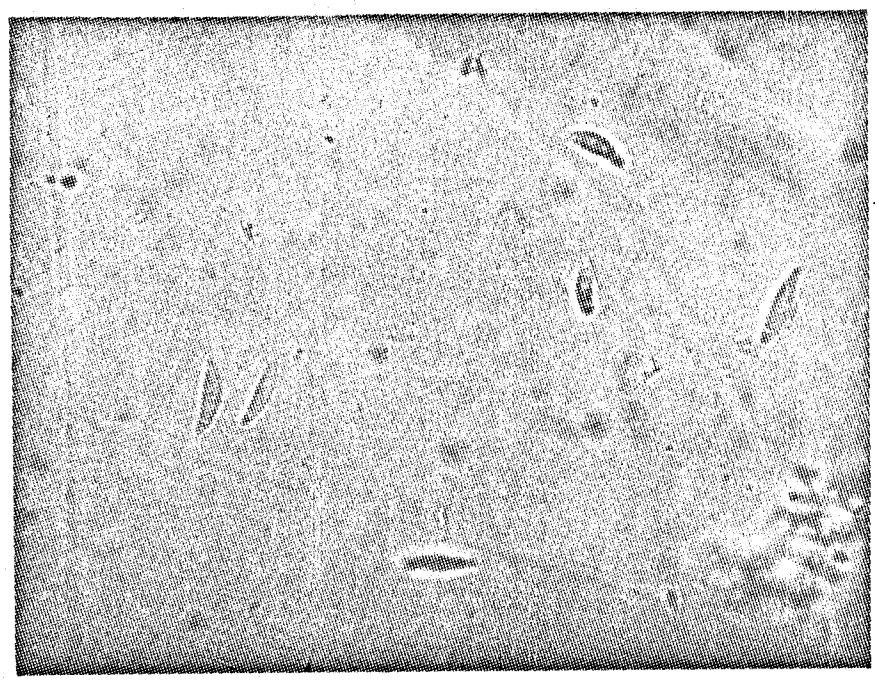


A



B

Fig. 2. Perithecia of Gnomonia veneta on overwintered leaves: (A) sycamore and, (B) white oak.



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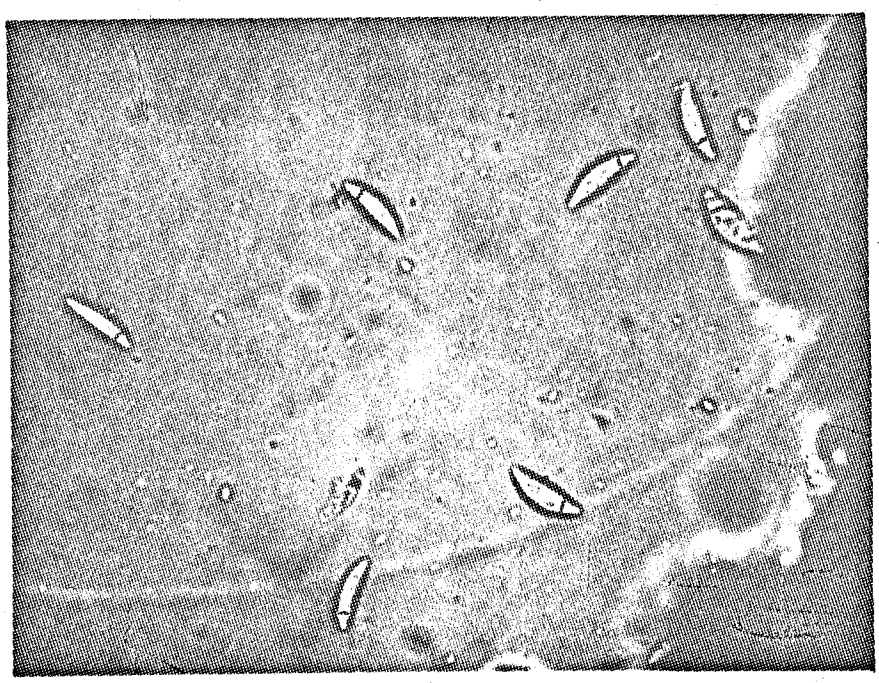


B

Fig. 3. Ascospores of Gnomonia veneta from perithecia produced on naturally infected overwintered leaves, (A) sycamore (B) white oak (675X).

A

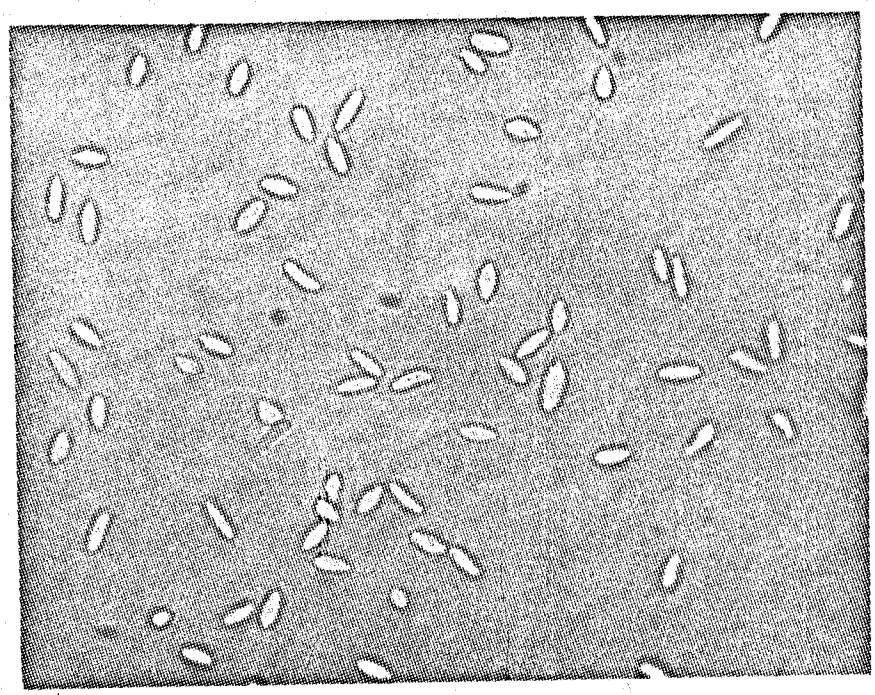
B

Fig. 4. Asci of Gnomonia veneta from perithecia produced on naturally infected overwintered leaves, (A) sycamore (B) white oak (675X).

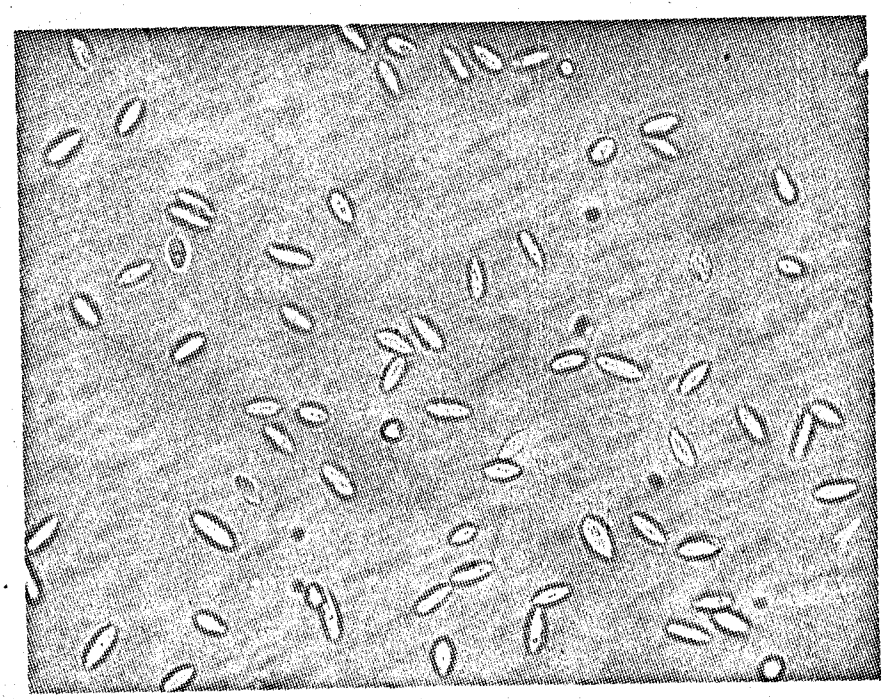
Comparisons of conidia from representative cultures obtained from single ascospores from white oak and sycamore tissue show little difference in size (Table 2). When these conidia are compared with those produced in cultures from naturally infected trees little difference is noted in appearance (Fig. 5 and 6) and spore size (Table 2). Furthermore, cultural characteristics of the sycamore group isolates and single ascospore isolates from oak and sycamore are similar.

In summary, conidia from ascospore and naturally infected tissue are indistinguishable, regardless of source. The ascogenous stages from white oak and sycamore do not differ except for perithecial beak length. The morphological and cultural evidence indicates that these isolates can all be included in Gnomonia veneta.

In contrast, the conidia from the oak group are distinctly different from those of the sycamore group. The spores ranged from 10 - 18 microns in length to 4 - 13 microns in width (Table 2). Besides being larger, the spores from the oak group were more granular and more uniformly shaped (Fig. 7, 6 and 5). When cultures from this group were incubated at optimum temperature on potato-dextrose agar plates, concentric rings of black spore masses 4 to 8 millimeters in width were produced with fine tufts of mycelium breaking through between the rings (Fig. 8A). While in sycamore group cultures the spores usually developed on raised concentric rings rather than between the tufted mycelial rings, and the sporulating surface was tan or yellow in color (Fig. 8B). The morphological and cultural evidence to date indicates the oak group is a distinct entity separate from Gnomonia veneta.

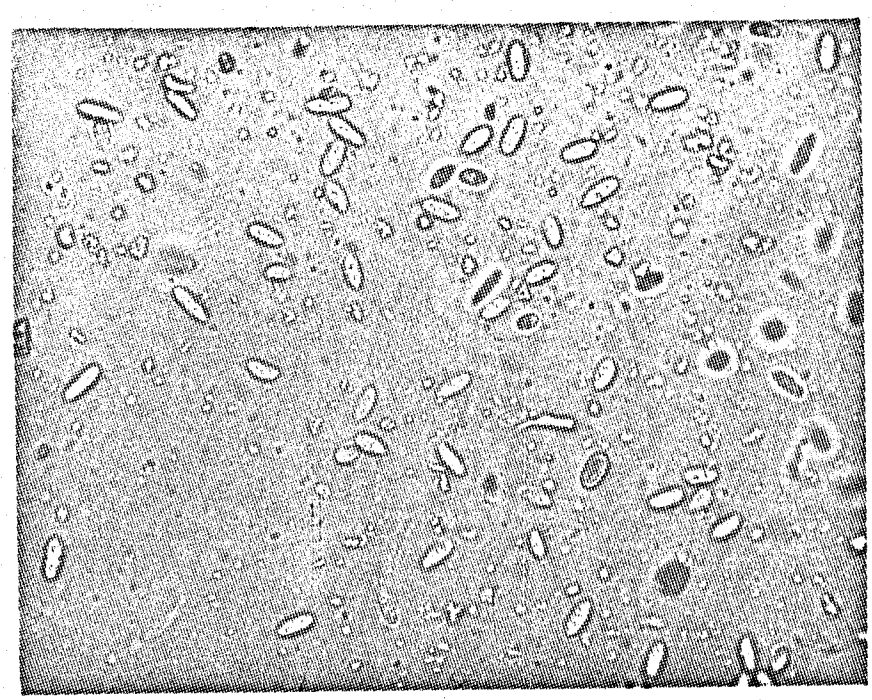


A

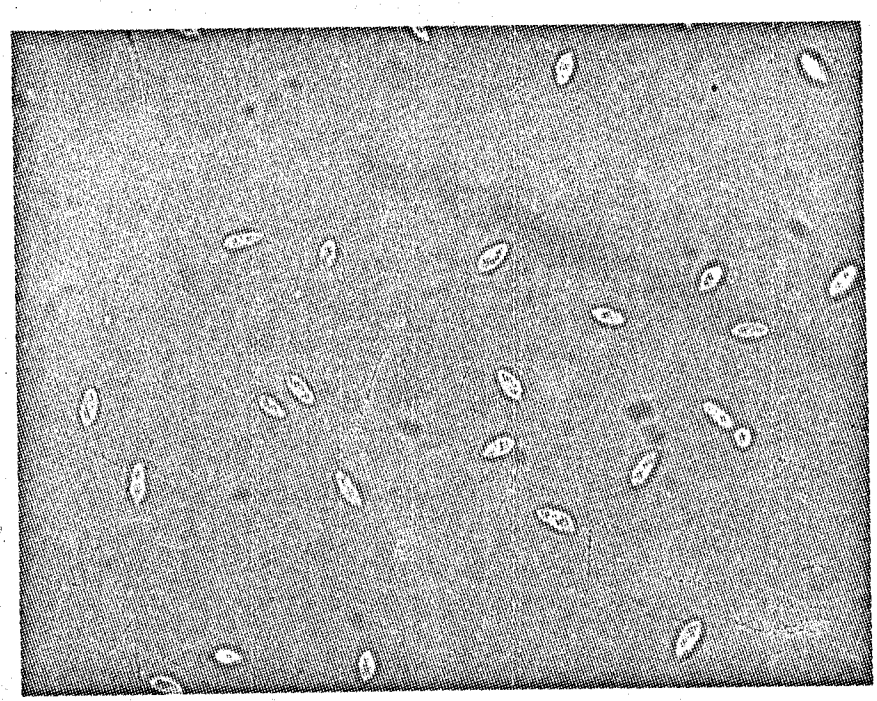


B

Fig. 5. Conidia from single ascospore isolates grown on potato-dextrose agar, (A) from perithecia on sycamore leaves, (B) from perithecia on oak leaves (450X).

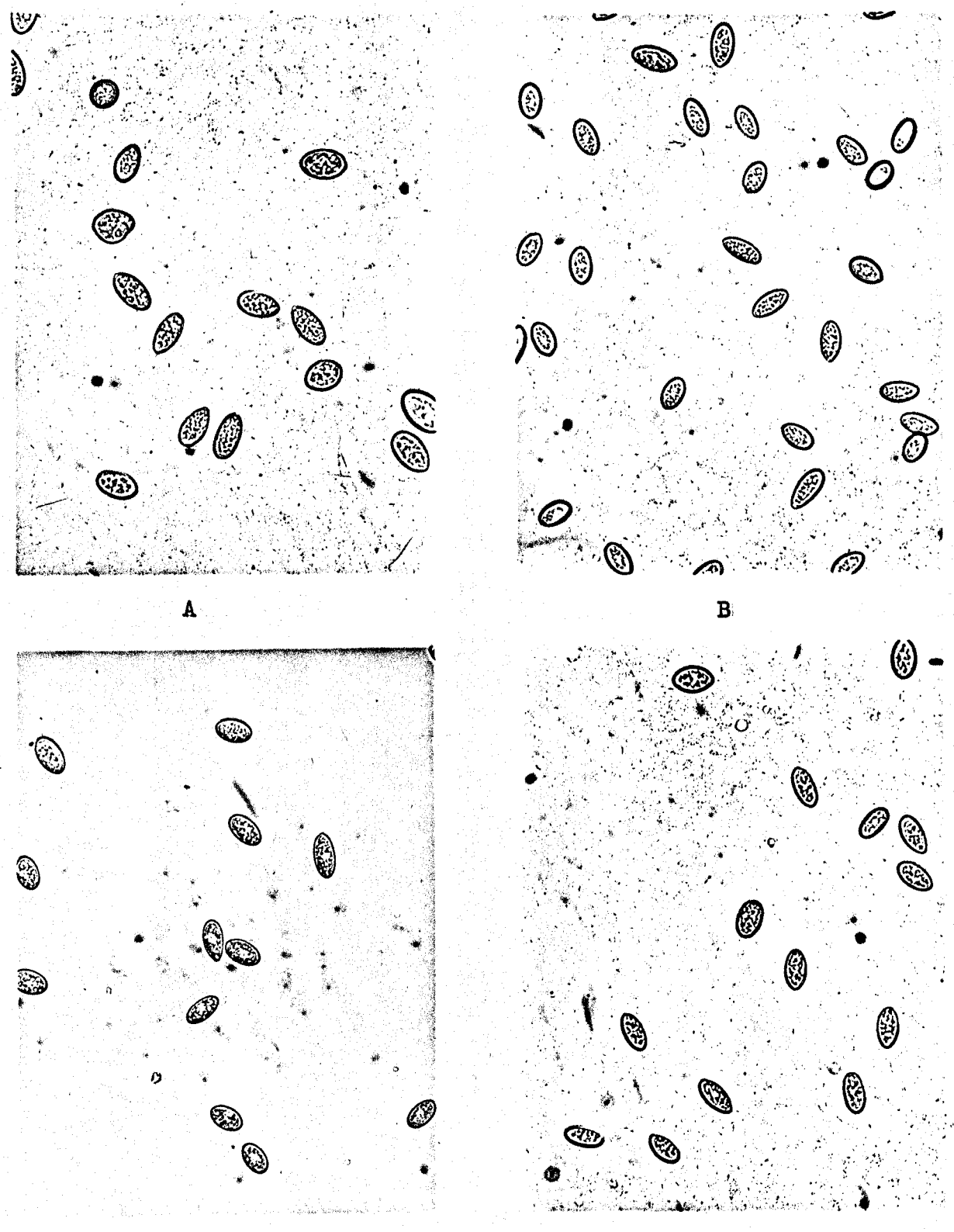


A



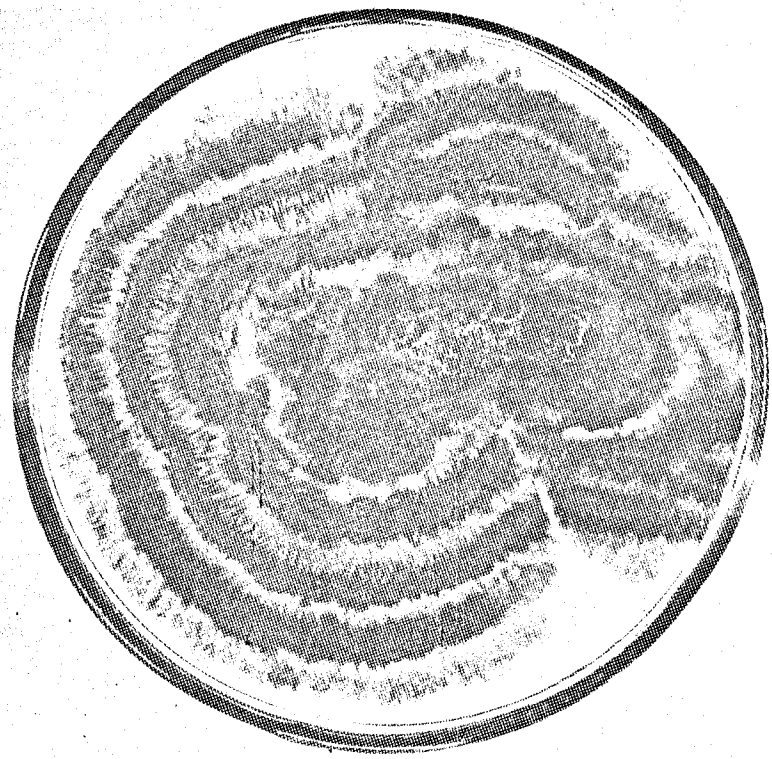
B

Fig. 6. Conidia of Gnomonia veneta (sycamore group) isolated from: (A) white oak (B) sycamore and grown on potato-dextrose agar (450X).

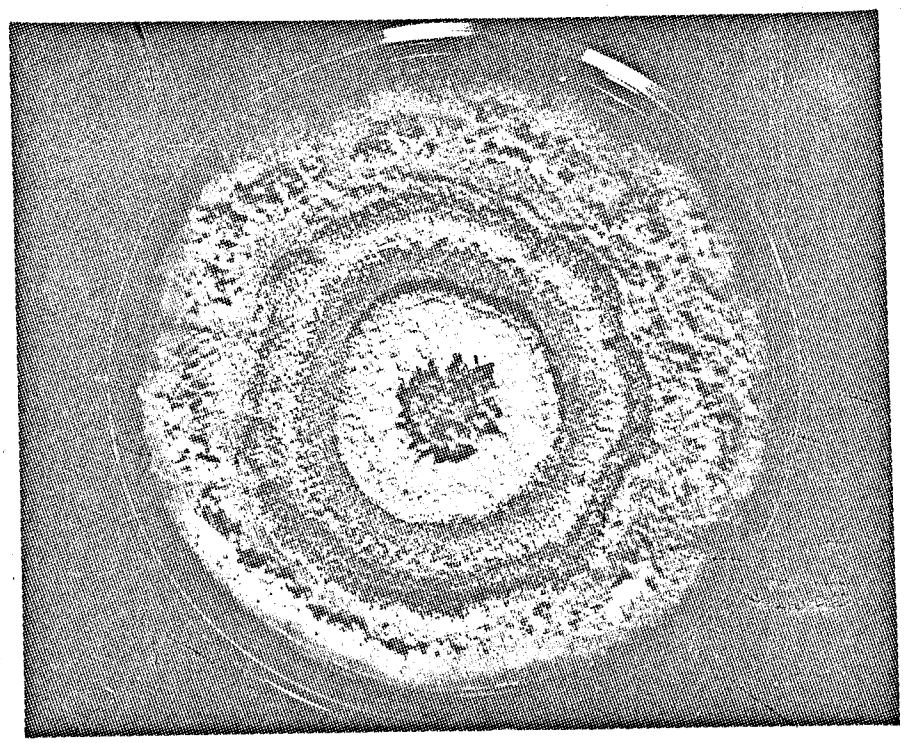


A B C D

Fig. 7. Conidia of the oak group isolated from: (A) black walnut, (B) white oak, (C) red oak and (D) American elm and grown on potato-dextrose agar (450X).



A



B

Fig. 8. Characteristic growth habit of anthracnose fungi isolated from (A) white oak (oak group) and (B) sycamore (sycamore group) grown on potato-dextrose agar.

Table 2. Comparison of conidia from different sources.

Code	Source of conidia	Length and width of 100 conidia	
		Range (μ)	Mean (μ)
		Sycamore group	
		Single ascospore cultures	
AO-4	White oak	7.9-13.0 x 2.9-5.8	10.2 x 4.7
SA-1	Sycamore	7.2-13.0 x 2.9-5.8	9.8 x 4.8
		Cultures from naturally infected trees	
G-2	Sycamore	5.8-13.0 x 2.9-6.5	9.3 x 5.1
MO-1-1	White oak	6.5-14.4 x 4.3-6.5	9.5 x 5.2
		Oak group	
		Cultures from naturally infected trees	
B-3-3	White oak	11.5-18.7 x 7.2-13.0	14.1 x 9.6
MA-2-3	Red oak	10.8-16.1 x 4.3-8.6	12.6 x 7.7
MW-1-1	Black walnut	11.5-18.7 x 6.5-10.8	15.0 x 8.2
KE-1-1	American elm	10.1-15.5 x 6.0-9.2	12.6 x 7.7

Effect of Growth Factors on Anthracnose Fungi from Oak and Sycamore

Failure of fungi to grown on synthetic media such as Czapek's or Elliott's agar is due in many instances to inability of the organism to synthesize one or more necessary growth factors. It was found that certain isolates of Gloeosporium from infected oak and sycamore failed to grow on synthetic media (29, 51). However, in a basal medium containing mineral salts, dextrose, trace elements and combinations of vitamins growth would occur.

In a preliminary test to determine which of ten growth factors had the greatest effect on the development of isolates from oak and sycamore, one was omitted in succession from each of ten treatments to which the other nine had been added. In addition, treatments included one with all and one with no amendments. In this test and other similar tests at least four replications of each treatment were used and the flasks, which contained 50 ml. of medium, were seeded with a uniform spore suspension. Growth was determined by filtering the mycelium on previously weighed filter paper, drying and weighing to the nearest milligram. The results show that when inositol or thiamin was omitted the growth of the isolate from the oak group (B-3-3) was greatly reduced. In contrast growth of the sycamore isolate (G-2) was reduced when thiamin or biotin was omitted (Table 3). No growth or only slight growth occurred when vitamins were not added.

Table 3. Effect of growth factors on Gloeosporium isolates from white oak and sycamore.

Nutrient substrate		Mean weight of mycelium	
Medium No.	Amendments to basal medium	B-3-3 (white oak) mg.	G-2 (sycamore) mg.
1	All supplements	69	86
2	All except thiamin	19	26
3	All except biotin	90	13
4	All except pyridoxine	121	86
5	All except inositol	16	60
6	All except PAB	113	74
7	All except calcium pantothenate	129	49
8	All except nicotinic acid	124	71
9	All except choline	54	43
10	All except riboflavin	103	42
11	All except folic acid	84	68
12	No amendments (check)	13	16

Growth factor requirements of isolates from the oak group

To determine if the agar plate is satisfactory for testing the growth factor requirements of these fungi, isolate B-3-3 from the oak group was grown on 2 percent agar containing the basal medium and combinations of growth factors. In two tests this isolate showed a complete deficiency for inositol and a partial deficiency for thiamin and choline (Table 4). Thiamin was observed to increase sporulation as well as growth. Comparable growth occurred with inositol and thiamin or inositol and choline, however, different results were obtained in liquid culture with these supplements. This illustrates the difficulty of determining accurately the amount of growth when only increase in colony diameter is measured, and when the type of growth on the agar suface, which varies with treatment, is disregarded. No growth occurred without vitamins indicating that agar does not contain growth factors in quantities large enough to sustain growth. The agar plate is useful in screening those vitamins which grossly affect growth but for accurate determinations liquid culture must be used.

Since Gloeosporium isolates of the oak group resemble each other in morphological and cultural characters, tests were undertaken to determine if isolates from different hosts have the same biochemical response. In comparable tests on isolate B-3-3 from white oak and AP-103 from red oak the responses were similar, with both isolates showing a complete deficiency for inositol and a partial deficiency for thiamin. When thiamin was omitted there was a greater response in the presence of inositol to choline and riboflavin than was observed with inositol alone. This was

Table 4. Effect of growth factors on Gloeosporium isolate (B-3-3) from the oak group growing on solid medium.

Nutrient substrate		Increase in colony diameter	
Medium No.	Amendments to basal medium	Test I mm./day	Test II mm./day
1	All supplements	6.6	6.5
2	Thiamin, inositol, choline	6.4	6.6
3	Thiamin, choline	0	-
4	Thiamin, inositol	4.1	4.5
5	Inositol, choline	4.1	4.6
6	Thiamin, biotin, choline, folic acid	0	-
7	Pyridoxine, PAB, calcium pentothenate, nicotinic acid, riboflavin	0	-
8	Inositol	-	4.0
9	Thiamin	-	0
10	Choline	-	0
11	Potato-dextrose agar	-	6.3
12	No amendments	-	0

especially evident for isolate AP-103 from red oak. The difference in the amount of growth of the two isolates is difficult to explain since the amount of inoculum added to each flask and incubation temperatures were similar (Table 5).

A third isolate of the oak group KE-1-1 from American elm was tested to determine if its biochemical response was similar to the other isolates of this group. As shown in table 6 this isolate had a complete deficiency for inositol and a partial deficiency for thiamin which is in agreement with data on the other isolates of the oak group.

More growth was recorded for isolates of the oak group when yeast extract was added to the basal medium than for any other combination of amendments. However, when vitamin-free casein hydrolysate, thiamin and inositol were added to the basal medium, which contained only an inorganic source of nitrogen, growth of isolate B-3-3 from the oak group equaled that obtained with yeast extract. This indicates inositol and thiamin are the important vitamins necessary for the growth of this isolate. It is also apparent this fungus cannot synthesize enough of the necessary proteins from an inorganic source of nitrogen for maximum growth. This experiment also illustrates the beneficial effect of shake culture. At least a two-fold increase in growth was obtained in 6 days in shake culture, as compared to 17 days in still culture (Table 7). Vitamin B-12 decreased the amount of growth when added with growth factor combinations of inositol and thiamin, or inositol, thiamin and casein hydrolysate (Table 7, media 4 & 6; 5 & 3).

Table 5. Effect of growth factors on two isolates from the oak group; B-3-3 from white oak and AP-103 from red oak.*

Nutrient substrate		Mean weight of mycelium	
Medium No.	Amendments to basal medium	B-3-3 (white oak) mg.	AP-103 (red oak) mg.
1	Yeast 0.4%	154.2	284.2
2	All supplements	69.7	185.6
3	No amendments	0	0
4	Thiamin, inositol, choline	86.6	145.6
5	Inositol, thiamin	88.6	141.4
6	Inositol, choline	40.0	23.4
7	Inositol, biotin	27.0	13.6
8	Inositol, folic acid	20.2	8.6
9	Inositol, pyridoxine	22.8	8.4
10	Inositol, riboflavin	26.6	21.6
11	Inositol	24.0	8.2
12	All supplements except inositol	0	3.2

* Period of growth 15 days

Table 6. Effect of growth factors on Gloeosporium isolate KE-1-1 from American elm (oak group).*

Nutrient substrate		Mean weight of mycelium
Medium No.	Amendments to basal medium	KE-1-1 (American elm) mg.
1	Yeast 0.4%	345.5
2	All supplements	74.5
3	No amendments	0
4	All supplements except inositol	0
5	Inositol	23.7
6	Inositol, thiamin	75.5
7	Inositol, thiamin, choline	80.3

* Period of growth 12 days

Table 7. Effect of growth factors on Gloeosporium isolate B-3-3 from white oak in still and shake cultures.

Nutrient substrate		Mean weight of mycelium B-3-3 (white oak)	
Medium No.	Amendments to basal medium	Shake culture* mg.	Still culture** mg.
1	Yeast extract 0.4%	271	105
2	All supplements except inositol plus casein hydrolysate	0	0
3	Inositol, thiamin, casein hydrolysate	278.5	116.7
4	Inositol, thiamin, B-12	102.0	62.0
5	Inositol, thiamin, casein hydrolysate, B-12	230.7	91.7
6	Inositol, thiamin	138.5	70.7
7	No amendments	0	0

* Period of growth 6 days
** Period of growth 17 days

Another experiment with isolate B-3-3 from the oak group was undertaken to determine what other amendments affect growth when added separately to the basal medium plus thiamin, inositol and casein hydrolysate. The following amendments were added separately to a replicated series of treatments: biotin, pyridoxine, folic acid, calcium pantothenate, nicotinic acid, choline, B-12 and riboflavin. Thiamin and inositol alone gave more growth than any of the other treatments including yeast extract. Riboflavin and biotin as well as other vitamins had an inhibitory effect on growth under the conditions of this experiment (Table 8).

In the presence of vitamin-free casein hydrolysate, thiamin and inositol, the growth of isolates of the oak group was greater than on a medium containing these two vitamins and only the inorganic nitrogen of the basal medium. To further investigate the nitrogen requirements of this group, isolate B-3-3 was tested to determine specifically what amino acids have the greatest effect on growth. Two liters of medium with the following constituents and concentrations per liter were prepared: KH_2PO_4, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.25 gm.; dextrose, 20.0 gm.; inositol, 40.0 mg.; thiamin, 0.2 mg. and trace elements as previously indicated. This medium was divided into 20 equal lots and to each of 17 lots was added 0.1 gm. of one of the following amino acids: alanine, phenyl-alanine, leucine, cysteine, asparagin, aspartate, histidine, glycine, cystine, tryptophan, lysine•HCL, methionine, glutamate, serine, threonin, arginine and isolucine. The three remaining lots received (1) yeast extract, 0.4 percent, (2) inorganic nitrogen KNO_3 and NH_3NO_3 at 1 gm. per liter

respectively, and (3) no additions. Four aliquots of each lot in 50 ml. Erlenmeyer flasks were seeded with 1/2 ml. of a uniform spore suspension, and dry weight determinations were made as previously described. The most growth, 70 mg., was obtained with yeast extract. This would be expected since yeast contains a rich complex source of organic nitrogen and vitamins. Of the amino acids tested the largest dry weights were obtained with alanine, 42.7 mg.; tryptophan, 42.2 mg.; and arginine, 41.3 mg.; as compared with 28.6 mg. for the inorganic nitrogen source. All of the other amino acids tested produced growth comparable to that obtained with the inorganic nitrogen source except cysteine where no growth occurred (Table 9). These data show that the nitrogen source for this isolate is not critical.

Since isolates of the oak group show a complete deficiency for inositol, tests were undertaken to study the response of isolate B-3-3 from white oak at varying concentrations of inositol. Thiamin was added to the basal medium and the concentration of inositol was varied from 10 to 60 ppm. in steps of 10 ppm. The fungus was grown in liquid culture, replicated four times, and growth determinations made as previously described. Over the concentration range, the fungus showed little difference in response except for a small decrease in growth at 60 ppm. indicating that an inositol concentration of 10 ppm. was adequate or in excess of the amount needed for growth (Table 10). However, in a second test, adequately replicated, using shake culture with vitamin-free casein hydrolysate and thiamin added to the basal medium, the following mean dry weights of mycelium were obtained at the concentrations indicated:

Table 8. Effect of growth factors on Gloeosporium isolate B-3-3 from the oak group.

Nutrient substrate		Mean weight of mycelium
Medium No.	Amendments to modified basal medium*	B-3-3 (white oak)
		mg.
1	Yeast extract	104.7
2	All supplements except inositol	0
3	No amendments	0
4	Inositol, thiamin (no casein hydrolysate)	70.7
5	Inositol, thiamin (1 gm./liter casein equivalent)	76.0
6	Inositol, thiamin	116.7
7	Inositol, thiamin, B-12	91.7
8	Inositol, thiamin, biotin	88.2
9	Inositol, thiamin, pyridoxine	94.7
10	Inositol, thiamin, folic acid	111.2
11	Inositol, thiamin, calcium pantothenate	98.5
12	Inositol, thiamin, nicotinic acid	99.7
13	Inositol, thiamin, choline	109.5
14	Inositol, thiamin, riboflavin	81.0
15	All supplements	106.0

* Basal medium plus 2 gm. (casein equivalent) per liter vitamin-free casein hydrolysate.

Table 9. Effect of yeast extract, amino-acids and inorganic nitrogen on the growth of isolate (B-3-3) from white oak.

Nutrient substrate		Mean weight of mycelium
Medium No.	Amendments added	B-3-3 (white oak) mg.
1	Yeast extract, 0.4%	70.0
2	Alanine	42.7
3	Tryptophan	42.2
4	Arginine	41.3
5	Glutamate	35.0
6	Serine	32.2
7	Aspartate	31.7
8	Asparagin	31.3
9	Inorganic nitrogen-KNO_3; NH_3NO_3; (1 gm./l)	28.6
10	Glycine	28.5
11	Histidine	27.7
12	Leucine	27.0
13	Methionine	26.0
14	Threonin	25.3
15	Lysine•HCL	22.3
16	Iso-lucine	22.2
17	Phenyl-alanine	22.2
18	Cystine	13.5
19	No additions	4.5
20	Cysteine	0.0

Table 10. Effect of inositol concentration on the growth of Gloeosporium isolate B-3-3 from white oak.

Nutrient substrate		Mean weight of mycelium
Medium No.	Inositol concentration ppm.	B-3-3 (white oak) mg.
1	0	0
2	10	111.7
3	20	102.0
4	30	101.5
5	40	106.6
6	50	105.0
7	60	88.3
8	All supplements except inositol	0

Inositol Conc. ppm.	Mean weight of mycelium mg.
10	252.0
1	66.0
0.1	7.7
0.01	0

This fungus is sensitive to inositol at 0.1 ppm. which is well within the range necessary for microbiological assay. Since no growth appears at all in the absence of inositol, this isolate probably would make a very satisfactory organism for assay tests.

Growth factor requirements of isolates from sycamore

For the sycamore isolates, screening tests on agar plates indicated biotin and thiamin had the greatest influence on growth. To determine the specific relationships of biotin, thiamin and other vitamins, isolate G-2 from sycamore was tested in liquid culture. The results show this isolate has nearly a complete deficiency for biotin and a partial deficiency for thiamin. Thiamin and biotin added together resulted in good growth. When thiamin was added alone or in combination with choline, riboflavin, calcium pantothenate or folic acid no growth occurred. However, when thiamin and inositol were added, about one-fourth as much growth resulted as with thiamin and biotin. This indicates either inositol or biotin must be present in the medium for growth to occur, however, unlike the isolates from the oak group, growth occurred without

the addition of inositol. Biotin, thiamin and calcium pantothenate gave more growth than any other combination (Table 11).

A similar growth factor response was obtained in another test with isolate G-2 from sycamore. When biotin and inositol were not present in the media no growth occurred. When biotin alone was not present and all other growth factors were added, only slight growth occurred. When thiamin and biotin were present together in the medium the mycelial weight was 115.4 mg., however, when inositol was added to this combination 89.2 mg. were obtained (Table 12, media 11 & 7). This decrease in growth when inositol is added to biotin and thiamin is difficult to explain on the basis of this test. When vitamin-free casein hydrolysate was added with biotin, thiamin and inositol to the basal medium, a mean mycelial weight of 146 mg. was obtained, compared to 129 mg. for yeast and 89 mg. for biotin, thiamin and inositol alone (Table 12).

Since sycamore isolates have similar morphological and cultural characteristics, another isolate from sycamore (CS-6-2) was tested. It exhibited similar growth requirements by showing a complete deficiency for biotin and partial deficiencies for thiamin and inositol. Biotin and thiamin in combination gave 119 mg. as compared to 91.6 mg. for inositol, biotin and thiamin (Table 13, media 9 & 6).

These isolates from sycamore grew when biotin alone was added to the basal medium. This growth factor specificity distinguishes the sycamore group from the oak group which grew only when inositol was added to the basal medium.

Table 11. Effect of growth factors on conidial isolate G-2 from sycamore.

Nutrient substrate		Mean weight of mycelium
Medium No.	Amendments to basal medium	G-2 (sycamore) mg.
1	Yeast 0.4%	125
2	All supplements	91.6
3	All supplements except biotin and thiamin	8.0
4	No amendments	3.0
5	Biotin	31.6
6	Biotin, choline	28.0
7	Biotin, riboflavin	28.0
8	Thiamin, choline	0
9	Thiamin, riboflavin	0
10	Thiamin, calcium pantothenate	0
11	Thiamin, inositol	20
12	Thiamin, folic acid	0
13	Biotin, thiamin	85.0
14	Biotin, thiamin, calcium pantothenate	101.0
15	Biotin, thiamin, choline	78
16	Biotin, thiamin, riboflavin	90

Table 12. Effect of growth factors on conidial isolate G-2 from sycamore.

Nutrient substrate		Mean weight of mycelium (G-2)
Medium No.	Amendments to basal medium	sycamore mg.
1	Yeast 0.4%	129.5
2	All supplements	128.7
3	All supplements, plus casein hydrolysate	145.7
4	All supplements except biotin	7.8
5	All supplements except inositol and biotin	2.5
6	All supplements except biotin, thiamin and inositol	3.2
7	Biotin, thiamin	115.4
8	Biotin, thiamin, inositol	89.2
9	Biotin, thiamin, inositol, calcium pantothenate	111.2
10	Biotin, thiamin, inositol, casein hydrolysate	146.4
11	Biotin, thiamin, inositol, casein hydrolysate, B-12	137.6
12	Casein hydrolysate	1.8

Table 13. Effect of growth factors on isolate CS-6-2 from sycamore.

Nutrient substrate		Mean weight of mycelium
Medium No.	Amendments to modified basal medium*	CS-6-2 (sycamore) mg.
1	Yeast 0.4% (no casein hydrolysate)	150.0
2	All supplements except inositol and biotin	4.5
3	All supplements except inositol	43.0
4	All except biotin	9.25
5	Inositol, biotin, thiamin, (no casein hydrolysate)	77.6
6	Inositol, biotin, thiamin	91.6
7	Inositol, biotin, thiamin, B-12	126.0
8	All supplements except thiamin	18.5
9	Biotin, thiamin	119.0
10	No amendments	2.5

* Basal medium plus 2 gm. (casein equivalent) per liter vitamin-free casein hydrolysate.

Summary of growth factor requirements

Since the growth factor requirements for two sycamore conidial isolates were the same, it seemed desirable to determine if the remaining representatives of the sycamore group had similar requirements. To make this determination the following isolates were tested: from white oak, conidial isolate MO-1-1, and ascospore isolates, AO-1, AO-3, AO-4, AO-6, AO-8; from sycamore, conidial isolate G-2 and ascospore isolates AS-1 and AS-2. In addition this test included three isolates from the oak group. On the basis of the previous response of the oak group and sycamore conidial isolates, the following growth factor combinations were added to the basal medium: (1) biotin and thiamin, (2) inositol and thiamin and (3) no additions.

The isolates of the oak group all gave the expected response by exhibiting a complete deficiency for inositol. It was also expected that all members of the sycamore group would respond uniformly by showing a complete deficiency for biotin, however, an unexpected response was observed. The isolate from white oak and the associated ascospore isolates grew without the addition of vitamins. On biotin and thiamin,and inositol and thiamin, they grew equally well. The sycamore conidial isolates and the corresponding ascospore cultures responded as expected by showing a complete deficiency for biotin (Table 14).

This test indicates that three distinct physiological strains of anthracnose fungi occur on oak and sycamore. Morphologically the sycamore group is uniform and includes white oak and sycamore conidial isolates

Table 14. Physiological differences in isolates of the oak and sycamore group as shown by their growth factor requirements.

Code	Host and type of isolate	Amendments to basal medium: Biotin and thiamin	Inositol and thiamin	No amendments
		Mean mycelial dry wt. mg.	mg.	mg.
	Oak Group			
B-3-3	white oak (conidial)	0	84.2	0
KE-1-1	American elm (conidial)	0	75.5	0
AP-103	red oak (conidial)	0	141.4	0
	Sycamore Group Strain 1			
MO-1-1	white oak (conidial)	41.0	46.5	33.0
AO-1	white oak (ascospore)	69.2	70.2	28.5
AO-3	white oak (ascospore)	72.0	57.5	34.2
AO-4	white oak (ascospore)	34.0	37.0	26.5
AO-6	white oak (ascospore)	34.0	42.0	28.2
AO-8	white oak (ascospore)	26.5	36.7	21.7
	Strain 2			
G-2	sycamore (conidial)	45	11.2	5.2
AS-1	sycamore (ascospore)	84.5	4.0	2.5
AS-2	sycamore (ascospore)	78.5	13.2	8.5

and ascospore isolates. Within this group two types can be distinguished as to their growth factor requirements; one from sycamore, the other from white oak. The third physiological group includes the isolates of the oak group.

Effect of Thiamin on Sporulation

In some of the preliminary screening tests to determine growth factor requirements it was observed that thiamin not only had an effect on growth but also on sporulation. To test the validity of these observations an experiment was undertaken with representative isolates from both the oak and sycamore group. Solid media were used with the following additions to the basal medium: (1) all supplements except thiamin; (2) thiamin, biotin and inositol; (3) yeast extract; and (4) no additions. Petri plates were centrally inoculated with a small droplet of spore suspension in quadruplicate lots using the following cultures; B-3-3 (white oak) from the oak group, MO-1-1 (white oak), AO-1 (ascospore isolate from white oak), and G-2 (sycamore) from the sycamore group.

Thiamin was found to increase the sporulation of all isolates tested. Isolate B-3-3 (white oak) and G-2 (sycamore) gave a very striking visual effect because of the contrast between the black coloration where abundant sporulation occurred in the presence of thiamin, and the light-colored feathery growth where little sporulation of these fungi occurred in the absence of thiamin (Fig. 9). The same effect occurred with the other isolates but was not as apparent (Fig. 10). Spore counts were made by

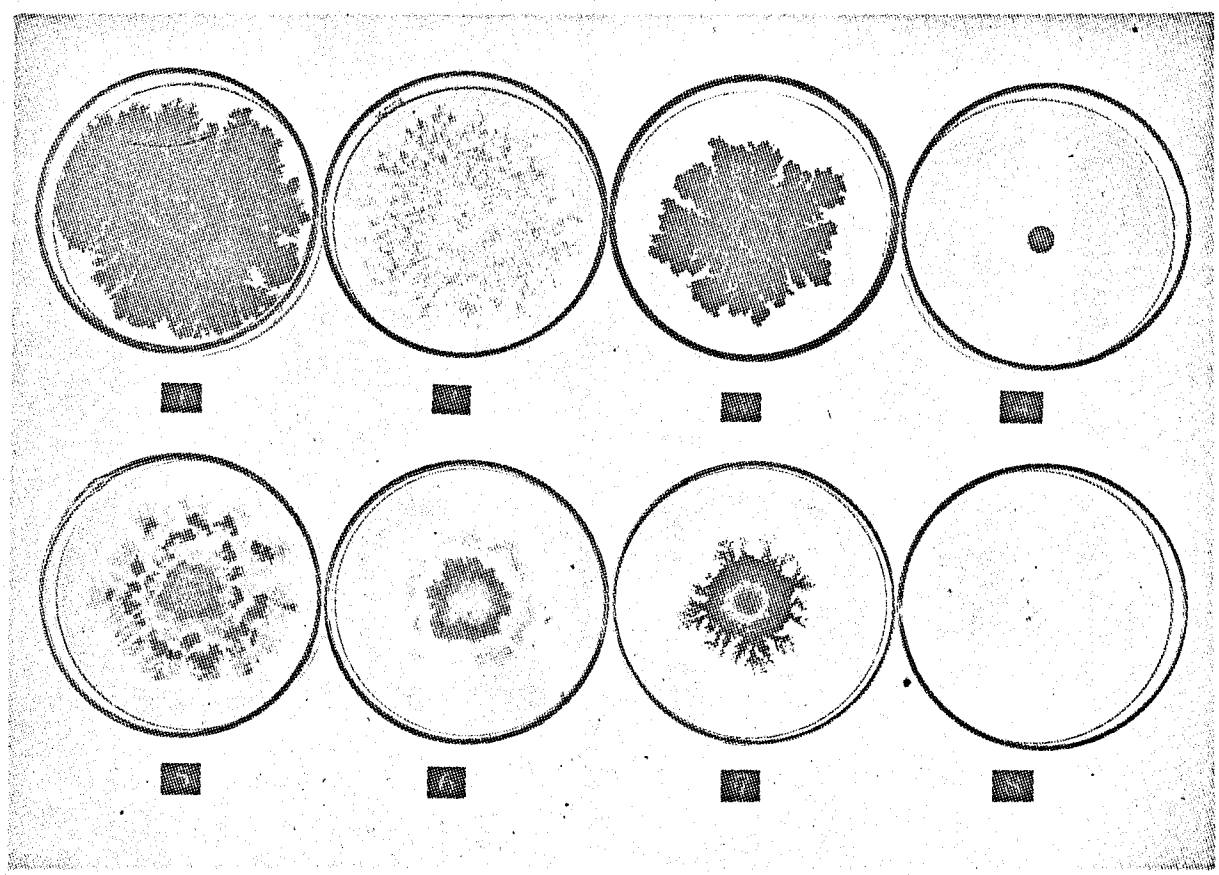


Fig. 9. Effect of thiamin on sporulation. Top row, isolate B-3-3 (white oak) of the oak group; below, isolate G-2 (sycamore) of the sycamore group. Both isolates were grown on 2 percent agar with the following additions to the basal medium: 1 and 5, yeast extract; 2 and 6, all supplements, except thiamin; 3 and 7, thiamin, biotin and inositol; 4 and 8, no additions.

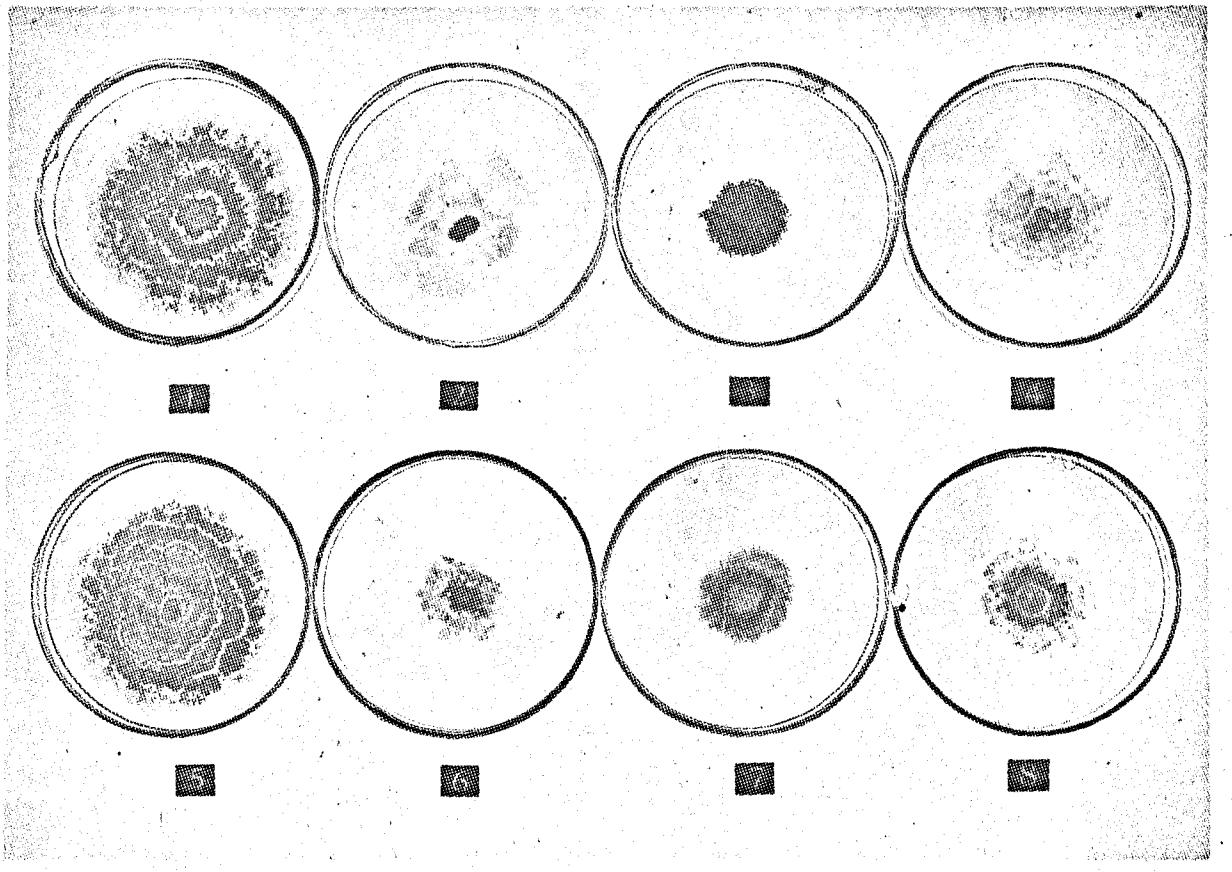


Fig. 10. Effect of thiamin on sporulation. Top row, isolate AO-1 (ascospore from white oak) of the sycamore group; below, isolate MO-1-1 (white oak) of the sycamore group. Both isolates were grown on 2 percent agar with the following additions to the basal medium: 1 and 5, yeast extract; 2 and 6, all supplements, except thiamin; 3 and 7, thiamin, biotin and inositol; 4 and 8, no additions.

removing from each plate the same amount of sporulating surface with its supporting agar to 10 ml. of water. The spores were then dislodged from the agar and the number of spores per milliliter determined with a counting chamber. An increase in sporulation of at least six fold was recorded for all isolates when thiamin, biotin and inositol were added to the basal medium as compared to all supplements except thiamin (Table 15). The sycamore group representative from white oak grew and sporulated without added vitamins (Fig. 10). In contrast, representatives of the other two strains did not even grow without the addition of vitamins (Fig. 9).

In the course of studies on the effect of thiamin and its relation to sporulation a peculiar phenomenon relating to the production of spores was observed. In a bacterial contaminated plate of isolate B-3-3 (oak group) spore production occurred abundantly only around the periphery of the contaminating colony. Since this occurred on a thiamin-free plate and thiamin increases sporulation, the bacterial contaminant apparently synthesized thiamin or some other material in sufficient quantities to promote sporulation of the fungus (Fig. 11).

Spore Germination and Inoculation Studies

Artificial infection has been difficult to achieve with conidial isolates from anthracnose infected white oak and sycamore. Some success was obtained by wounding the leaves or by removing the pubescence, and applying a spore suspension on the leaf surfaces with a camel's hair

Table 15. Effect of thiamin on the sporulation of isolates from the oak and sycamore groups.

Code	Host and type of isolate	Amendments to the basal medium: All supplements except thiamin	Thiamin, biotin, inositol	No amendments	Yeast
		Mean number of spores per ml. x 1000			
		Oak group			
B-3-3	white oak (conidial)	41.5	280	no growth	878
		Sycamore group			
MO-1-1	white oak (conidial)	149.5	1407	204	886
AO-1	white oak (single ascospore)	238.5	1008	216.5	1512
G-2	sycamore (conidial)	29.0	530	slight growth	650

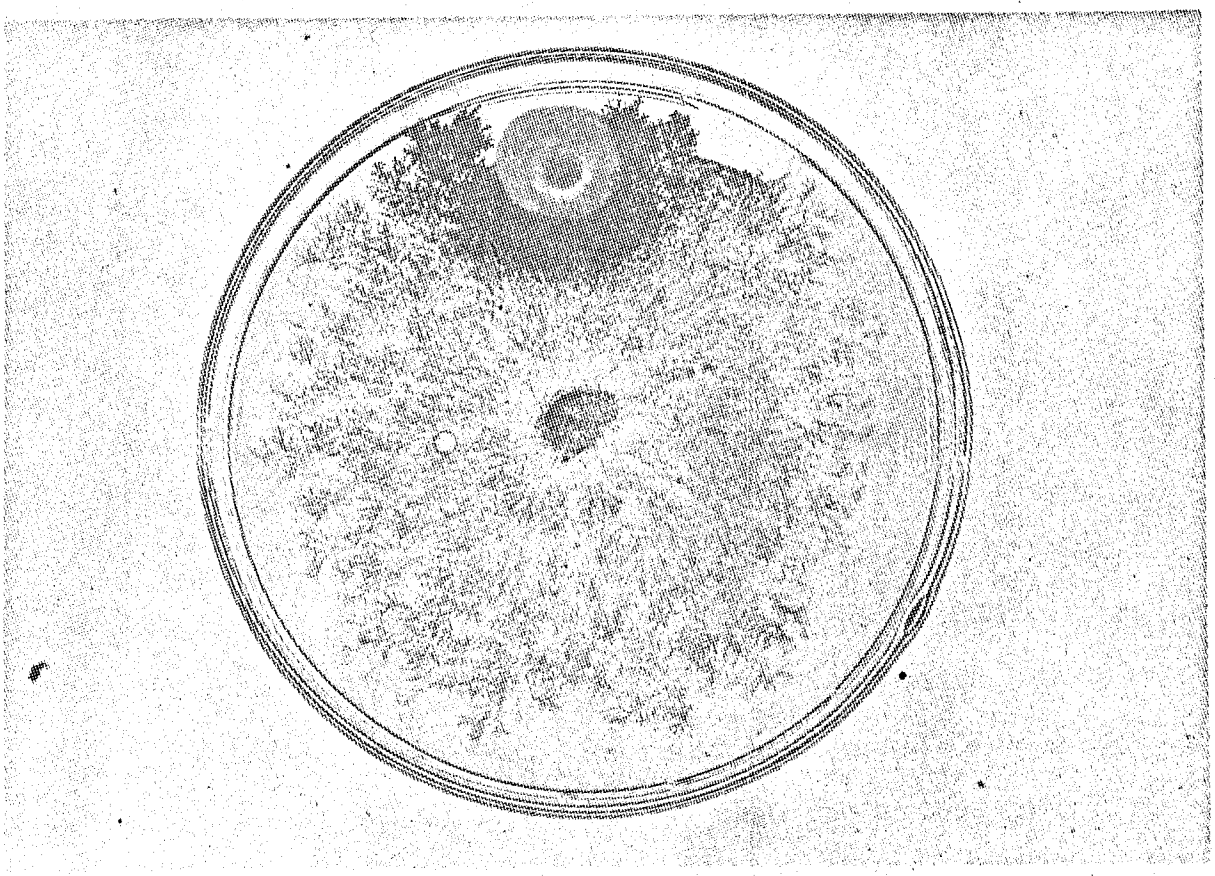


Fig. 11. Gloeosporium isolate B-3-3 (oak group) fruiting in the vicinity of a bacterial contaminant.

brush before putting the plants in the moist chamber. A spore suspension atomized on the leaf surfaces without removing the pubescence or wounding the leaves did not produce infection when 100 percent relative humidity was maintained in the moist chamber. However, in some experiments when a wetting agent such as sodium oleate was incorporated into the spore suspension infection occurred. Even better results were obtained with this wetting agent and a gradual reduction of humidity in the moist chamber.

Effect of sodium oleate and fatty acids on spore germination

To investigate the above phenomena the following spore germination studies were undertaken. A fresh 1 percent stock solution of sodium oleate was diluted to a final concentration of 0.5 percent with a spore suspension of isolate B-3-3 from the oak group; when tested by the glass slide method, no germination occurred. However, when this same stock solution was tested several days later the spore germination count increased two or three fold over the water checks. It was observed that the stock solution had changed from a clear solution to milky white. This suggested that oleic acid may be the active material causing the stimulatory effect. A series of tests using isolate B-3-3 from the oak group revealed that a 0.5 percent concentration of oleic acid, linoleic acid and sodium oleate adjusted with HCl from pH 10 to 7.5 or lower, stimulated spore germination at least two fold over the water checks. When a fresh sodium oleate solution (pH 10.3) was prepared

and tested no germination occurred (Table 16). A 2 percent dextrose solution usually increased spore germination over the water checks but the results were variable.

Isolates B-3-3 (white oak) and AP-103 (red oak) of the oak group were tested in a 0.5 percent solution of adjusted sodium oleate pH 7.5, dextrose and dextrose plus oleate. Both isolates responded the same way; the adjusted sodium oleate caused a 66 percent increase over the water checks for isolate B-3-3, and a 41 percent increase for isolate AP-103 from red oak (Table 17).

When identical tests were used on isolates AS-22-2 and SO-5 from sycamore a similar response was obtained. The adjusted sodium oleate caused a 46 percent increase over the water checks for isolate AS-22-2 and a 27 percent increase for isolate SO-5 (Table 18). These tests were repeated several times and increased spore germination always occurred with adjusted sodium oleate, oleic acid and linoleic acid.

Effect of spore germination stimulants on respiration

The effect of oleic acid, linoleic acid, sodium oleate and dextrose on the respiratory rate of isolate B-3-3 from the oak group was determined in the Warburg constant volume respirometer. One milliliter of spore suspension was added to each flask, and the proper amounts of the above materials were added to give the following final concentrations: 2 percent for dextrose, and 0.5 percent for the unsaturated fatty acids and the sodium oleate.

Table 16. Effect of unsaturated fatty acids, sodium oleate and dextrose on the germination of Gloeosporium isolate B-3-3 from white oak.

Treatment	Replicate number 1	2	3	4	5	6	Average
	Percent germination						
Check	30	42	23	43	41	25	34.0
Oleic acid 0.5%	88	84	83	91	85	91	87.0
Linoleic acid 0.5%	81	63	59	79	81	64	71.2
Dextrose 2%	45	34	29	30	40	38	36.0
Sodium oleate-0.5%, adjusted to pH 7.5	59	75	77	72	69	58	68.3
Sodium oleate-0.5%, pH 10.3	0	0	0	0	0	0	0

Table 17. Percentage spore germination of two isolates from the oak group as affected by 0.5 percent sodium oleate (adjusted to pH 7.5) and 2.0 percent dextrose.

Treatment	Replicate number						Average
	1	2	3	4	5	6	
	Percent germination						
	Isolate B-3-3 white oak						
Check	15	28	15	20	22	18	19.7
Na. Oleate	84	85	84	85	92	89	86.5
Dextrose	51	45	39	59	35	63	48.7
Na. Oleate plus dextrose	72	63	74	69	69	67	69.0
	Isolate AP-103 red oak						
Check	6	8	4	7	5	4	5.7
Na. Oleate	45	50	50	40	43	57	47.5
Dextrose	3	5	3	3	8	3	4.1
Na. Oleate plus dextrose	48	53	55	43	57	55	51.8

Table 18. Percentage spore germination of conidial isolates from sycamore as affected by 0.5 percent sodium oleate (adjusted to pH 7.5) and 2.0 percent dextrose.

Treatment	Replicate number 1	2	3	4	5	6	Average
	Percent germination						
Isolate AS-22-2 (sycamore)							
Check	16	14	14	18	15	20	16.1
Na. Oleate	78	70	68	45	54	61	62.7
Dextrose	18	28	25	24	18	25	23.0
Na. Oleate plus dextrose	65	78	76	74	71	76	73.3
Isolate SO-5 (sycamore)							
Check	4	5	3	4	3	2	3.5
Na. Oleate	32	39	29	24	21	39	30.7
Dextrose	3	4	4	4	2	1	3.0
Na. Oleate plus dextrose	23	28	34	37	33	23	29.7

The results of two representative experiments show linoleic acid, oleic acid and adjusted sodium oleate increased the O_2 uptake by 350 - 400 percent. Dextrose and unadjusted sodium oleate doubled the respiration rate. The fatty-acids plus dextrose did not give an additive effect (Table 19). A striking correlation exists between the increases in respiration and germination caused by these materials.

Effect of hydrogen-ion concentration on spore germination

Tests were made on isolate B-3-3 from the oak group using the glass slide method with vasoline rings to determine the optimum hydrogen-ion concentration for spore germination over a range of pH 3 to 8. Optimum germination occurred at pH 4 to 5 (Table 20). A distilled water check at pH 5.2 showed that the citric acid-phosphate buffer, diluted with four volumes of water, had little if any effect on germination other than that associated with change in hydrogen-ion concentration.

Effect of relative humidity and water film on spore germination

The effect of relative humidity on spore germination was determined for a representative isolate (B-3-3) of the oak group. Using the glass slide vasoline ring method, six uniform droplets of spore suspension were put on each slide and allowed to dry until free water had evaporated. One slide was then placed into each of six chambers at relative humidities of 95, 96, 97, 98, 99 and 100 percent. After incubation for 24 hours, none of the spores germinated.

Table 19. Effect of unsaturated fatty acids, sodium oleate, and dextrose on the respiratory rate of Gloeosporium isolate B-3-3 from white oak.

Treatment	Experiment I		Experiment II	
	Q_{O_2}*	T/C**	Q_{O_2}	T/C
Check	3.48		2.92	
Dextrose 2.0%	8.50	2.44	5.6	1.91
Oleic acid 0.5%	11.38	3.27	11.58	3.96
Linoleic acid 0.5%	12.10	3.48	11.19	3.82
Dextrose 2.0% plus Oleic acid 0.5%	12.1	3.48	9.95	3.40
Dextrose 2% plus Linoleic acid 0.5%	14.1	4.05	12.82	4.4
Na. Oleate 0.5% pH 10.3	7.34	2.10	5.65	1.93
Na. Oleate 0.5% adj. pH 7.5	11.9	3.42	11.5	3.94
Na. Oleate 0.5% adj. pH 3.5	9.2	2.64	9.75	3.34

* Q_{O_2} = μ liters/hr./mg. dry wt.
** T/C = treatment/check ratio

Table 20. Effect of pH on the germination of conidia from Gloeosporium isolate B-3-3 from white oak.

pH	Replicate number						Average
	1	2	3	4	5	6	
	Percent germination						
3	31	18	36	22	25	39	28.5
4	32	42	38	57	55	43	44.5
5	35	33	28	54	45	40	38.3
6	9	5	11	11	9	7	8.7
7	1	0	2	3	0	1	1.3
8	0	0	1	0	0	1	0.3
5.3 Dist. H_2O Check	39	56	38	41	40	41	42.5

In a similar test the uniform droplets of spore suspension were not pre-dried, but placed in the chambers immediately. The drying rate of the droplets increased with decreasing relative humidities. After 24 hours at room temperature, the droplets at 100 percent relative humidity were unchanged, while those in the 95 percent chamber had evaporated. Germination of 91.3 percent occurred in the 97 percent chamber, 46 percent in the 100 percent chamber and 54.3 percent in the 95 percent chamber (Table 21). This test was repeated three times with similar results. The thickness of water film over the spore surface and the length of time it exists is critical. This may help to explain why climatic conditions greatly influence the severity of anthracnose infection in the field.

Inoculation studies

Since oleic acid and the water film stimulates spore germination, attempts were made to use this information in inoculation studies. Oleic acid was incorporated into the spore suspension as a spore germination stimulant and wetting agent, and the relative humidity was reduced gradually in the moist chamber. The air in the incubation chamber was maintained at saturation by a fine water mist from a spray nozzle. Plants atomized with a spore suspension were put immediately into the chamber and after two or three hours the water mist was turned off. With the fan still operating to circulate the air in the chamber, the droplets on the leaf surfaces were gradually dried down. While this procedure was not entirely satisfactory it approached the conditions of the slide germination tests in the laboratory.

Table 21. Effect of decreasing relative humidities on the germination of spores in droplets of water (isolate B-3-3 from white oak).

Relative humidity	Replicate number						Average
	1	2	3	4	5	6	
	Percent germination						
100	54	44	51	45	34	48	46.0
99	50	57	61	67	67	67	61.5
98	74	71	70	69	60	76	70.0
97	89	92	94	93	88	92	91.3
96	86	81	85	84	86	93	85.8
95	45	52	63	53	59	54	54.3

To determine the effect of oleic acid (0.5 percent final concentration) and the rate of drying on infection, one-year-old white oak seedlings with mature and young leaves were sprayed with a conidial suspension of isolate B-3-3 from the oak group. The following seven treatments were replicated three times; results were recorded ten days after inoculation.

Treatment	Percentage of leaf surface destroyed by disease
Young leaves	
1. Spore suspension in water	35
2. Spore suspension in oleic acid	75
3. Check, oleic acid in water	0[1]
Mature leaves	
4. Spore suspension in water	0
5. Spore suspension in oleic acid	60
6. Check, oleic acid in water	0
7. Check, sprayed with water only	0

Oleic acid and a gradual drying of the spore suspension on the leaf surfaces encouraged infection and can be explained by the spore germination studies. Oleic acid was phytotoxic to young leaves but had no noticeable effect on mature leaves. The fungus was reisolated from infected leaves. Other experiments on white oak seedlings with this

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[1] Some injury due to oleic acid on leaf margins

isolate gave similar results. Attempts to infect white oak with isolate G-2 from sycamore by this method were successful on a few trees.

By using oleic acid (0.25 percent), the gradual drying method, removal of pubescence and by applying spore suspensions on leaf surfaces with a camel's hair brush, inoculations were successful in a few cases on both white oak and sycamore trees with a conidial isolate from sycamore. More tests are needed under accurately controlled conditions of temperature and relative humidity to determine the combined and separate effect of these factors on infection.

Studies on the Life Cycle of Anthracnose Fungi

The principal source of inoculum for early spring infection on sycamore is not the ascospore stage, but the conidial stage on the dead twigs. On Polk Boulevard in Des Moines, Iowa, where a spray program was conducted on sycamores, severe spring infection occurred, although all old leaves had been removed from under the trees. Repeated attempts to find the perfect stage on overwintered leaves in nature have been unsuccessful during the course of this study.

Viable conidia were found in the fall and early spring in the acervuli on dead sycamore twigs. Isolations from dead twigs have been successful throughout the winter, but repeated isolations from the buds of white oak and sycamore from February to April have been unsuccessful. In an attempt to follow the movement of the fungus through the host tissue, isolations were made from the following infected tissues after leaf blight

symptoms began to appear: leaf blade, midvein, leaf petiole, leaf trace and two inches below the node where the blighted leaf was attached. The twigs from which these isolations were made, outwardly appeared healthy; however, brownish, discolored streaks which followed the leaf trace and continued down into the woody tissue between the nodes were frequently found. The fungus was recovered from each of these areas in about 60 percent of the attempts. Free-hand sections from apparently healthy petioles of infected leaves showed the fungus in the vessels (Fig. 12). This indicates the fungus grows from the blade through the vessels of the petiole into the twig and may cause twig blight.

Cankers are formed on larger twigs and branches as the fungus grows in the host tissue. When longitudinal sections are made through cankers, brown discolored tissue is observed in the periphery of the cankered area. Attempted isolations from the periphery of cankers on one-year-old twigs have been successful, but in clear, healthy areas below the canker the fungus was not recovered by isolation. Attempted isolations from petioles of sycamore leaves which appeared to be healthy have been successful.

To determine if new growth can become infected from a canker and to prevent infection from airborne spores, glassine bags were put over healthy twigs adjacent to a cankered twig when the buds were dormant. In May when the disease became apparent, the leaves in some of the bags, although not infected from an outside source, showed typical anthracnose symptoms and fruiting bodies of the fungus (Fig. 13).

In the greenhouse one-year-old sycamore seedlings were inoculated with conidial and ascospore isolates. The spore suspensions were injected into the one-year-old wood at the base of new growth. About two weeks after injection, disease symptoms appeared on the leaves of the new growth and several days later the fungus began to form small cankers and fruiting bodies on the dead tissue (Fig. 14). This method of inoculation was successful in about half of the attempts and provides added evidence that the fungus can grow from twigs to leaves. Repeated attempts to infect white oaks by this method have not been successful although twig infection occurs on this host.

Control of Sycamore Anthracnose

Sycamore anthracnose belongs to a group of plant diseases with life cycle patterns which suggest the use of fungicidal sprays for their control. In 1950 spray experiments at Des Moines, Iowa, were conducted on Polk Boulevard to test the effectiveness of five fungicides, and on Thompson Avenue to determine what combination of sprays corresponding to progressive stages of development of the new leaves is necessary to control sycamore anthracnose with phenylmercuri-triethanol ammonium lactate.

All of the materials used on Polk Boulevard decreased the prevalence of infection (Table 22). The difference between the unsprayed checks and the various treatments was significant at the 5 percent level, but differences between treatments were not significant (Table 23). The treatments in decreasing order of apparent effectiveness were: Bordeaux mixture,

tribasic copper sulfate (Tennessee Tribasic Copper Sulphate), 2,3-dichloro-1,4-naphthaquinone (Phygon), ferric dimethyldithiocarbamate (Fermate) and phenylmercuri-triethanol ammonium lactate (Puratized Agricultural Spray) (Fig. 16). These small differences may well have been due to experimental error; however, under the conditions of moderate natural infection which existed, and on the basis of two years' results Bordeaux mixture seems a very satisfactory material for controlling sycamore anthracnose. There was a significant interaction between reading date and treatment. The incidence of disease varied greatly with the reading date in the check, but this variation was reduced with the better chemicals (Fig. 17).

On Thompson Avenue the treatments do not appear significantly different by a standard analysis of variance test (Tables 24 and 25). However, by use of a "t" test to compare the means of groups of treatments, the combination of spray 1 and 2 and 1, 2 and 3 were significantly better than all other treatments at the 1 percent level. This comparison is justified since a dormant spray with eradicant action to destroy the fungus spores on the dead twigs, followed with a protective spray on the young leaves as they emerge, would be expected to control the disease. Phenylmercuri-triethanol ammonium lactate has both of these properties and a combination of spray 1 and 2 had the greatest effect of all treatment combinations used (Fig. 18).

Phenylmercuri-triethanol ammonium lactate was the only chemical of the five tested that caused damage to leaf surfaces (Fig. 19). Injury was most severe on the smaller trees on Polk Boulevard but of little consequence on the large trees on Thompson Avenue. This may be explained

by the fact that the smaller trees received proportionately greater amounts of spray with greater force since the foliage was nearer the nozzle. Due to this injury on Polk Boulevard, it was comparatively difficult to make accurate disease readings on trees sprayed with this chemical; hence they are not included in the analysis.

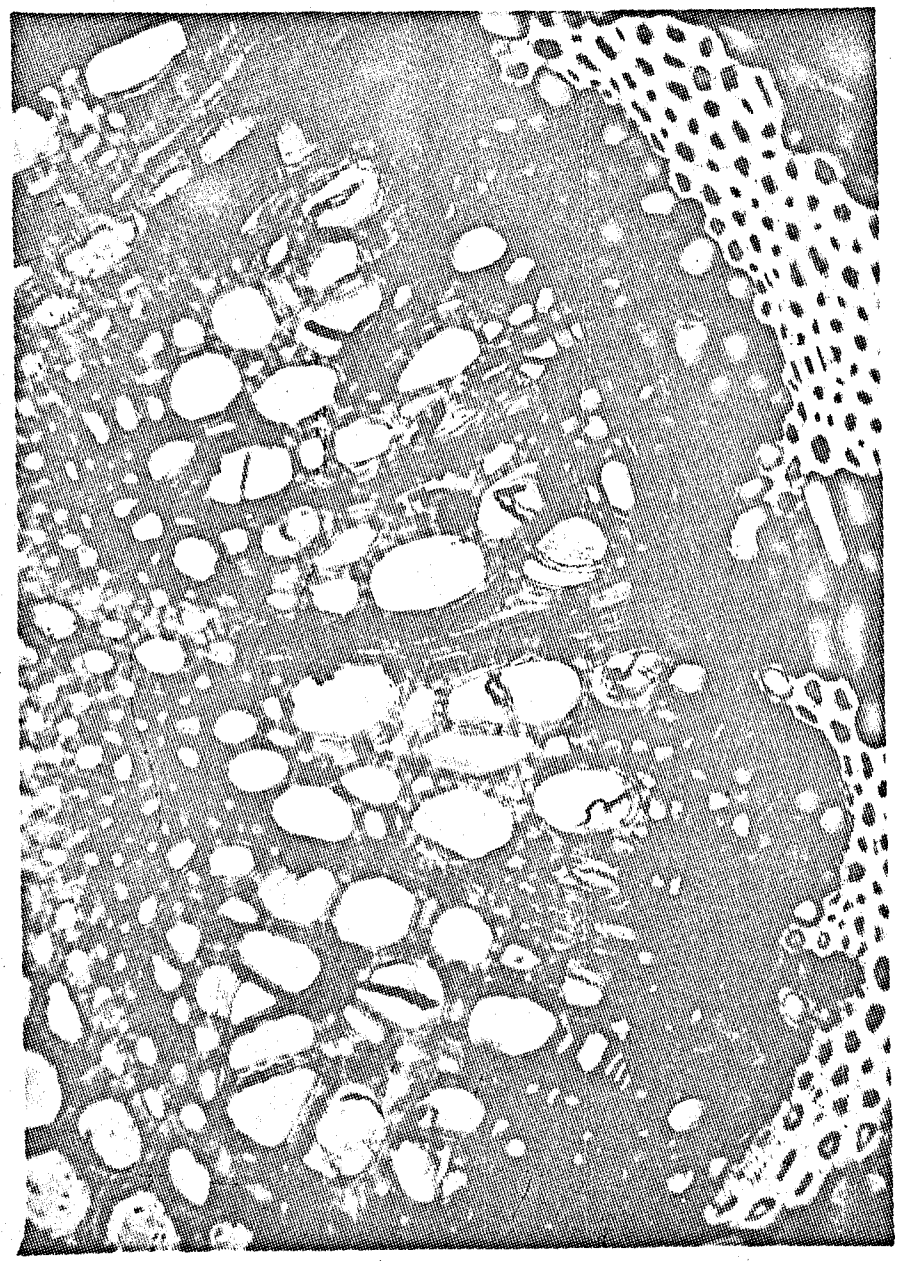

Fig. 12. Cross section of an apparently healthy sycamore petiole showing mycelium in the vessels (300X). The leaf blade was diseased.

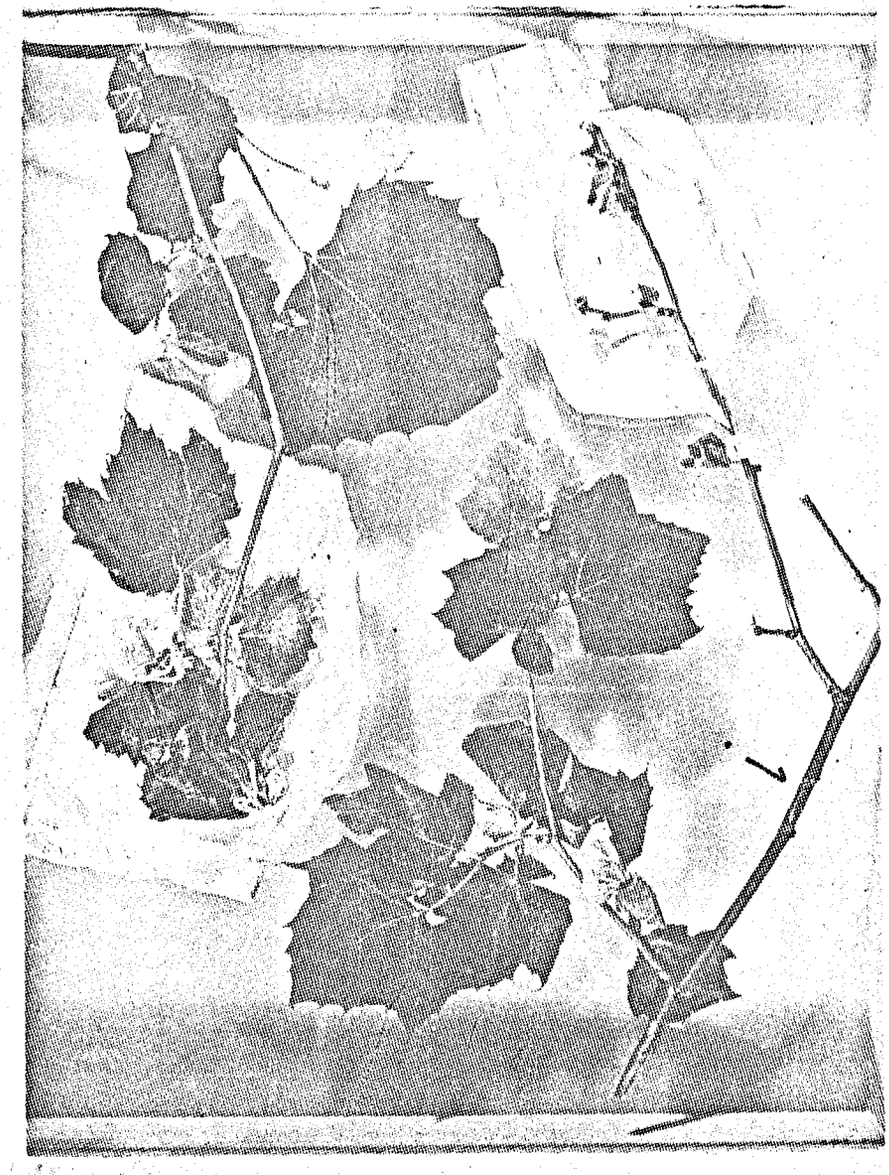

Fig. 13. Diseased sycamore leaves and twigs which probably became infected from an adjacent stem canker (arrow) while enclosed in glassine bags to protect against infection from an outside source.

Fig. 14. Symptoms and fruiting structures of anthracnose on current season's growth of a one-year-old sycamore seedling inoculated by injecting a spore suspension of Gnomonia veneta into the stem (arrow).

ing sycamore anthracnose on Polk Boulevard

plication on three June reading dates*										Mean infection per tree
	IV			V			VI			
20	3	8	20	3	8	20	3	8	20	
23	5	35	32	28	28	40	1	26	33	18
18	22	57	21	35	245	87	59	55	58	25
48	3	64	42	55	90	107	12	30	73	35
78	18	44	39	37	66	82	11	170	140	40
170	160	270	170	165	670	271	15	220	116	118

Table 23. Analysis of variance for effectiveness of four fungicides in controlling sycamore anthracnose on Polk Boulevard.

Sources of variation	Degrees of freedom		Mean square	F
Dates (D)	2		47,769	
Trees	89			
Treatments (T)		4	88,997	
Check vs. treatments		1	340,587	8.12*
Among treatments		3	15,400	
Replication (R)		5	40,904	
T X R (Error)		20	13,765	
Error (check vs. treatments)		5	41,954	
Error among treatments		15	13,108	
Trees in T X R		60	5,367	
Dates x trees	178			
D x T		8	14,597	4.79**
D x R		10	5,515	
D x T x R (Error)		40	3,044	
Observations in D x trees		120	2,659	

* Indicates significance at the 5 percent level
** Indicates significance at the 1 percent level

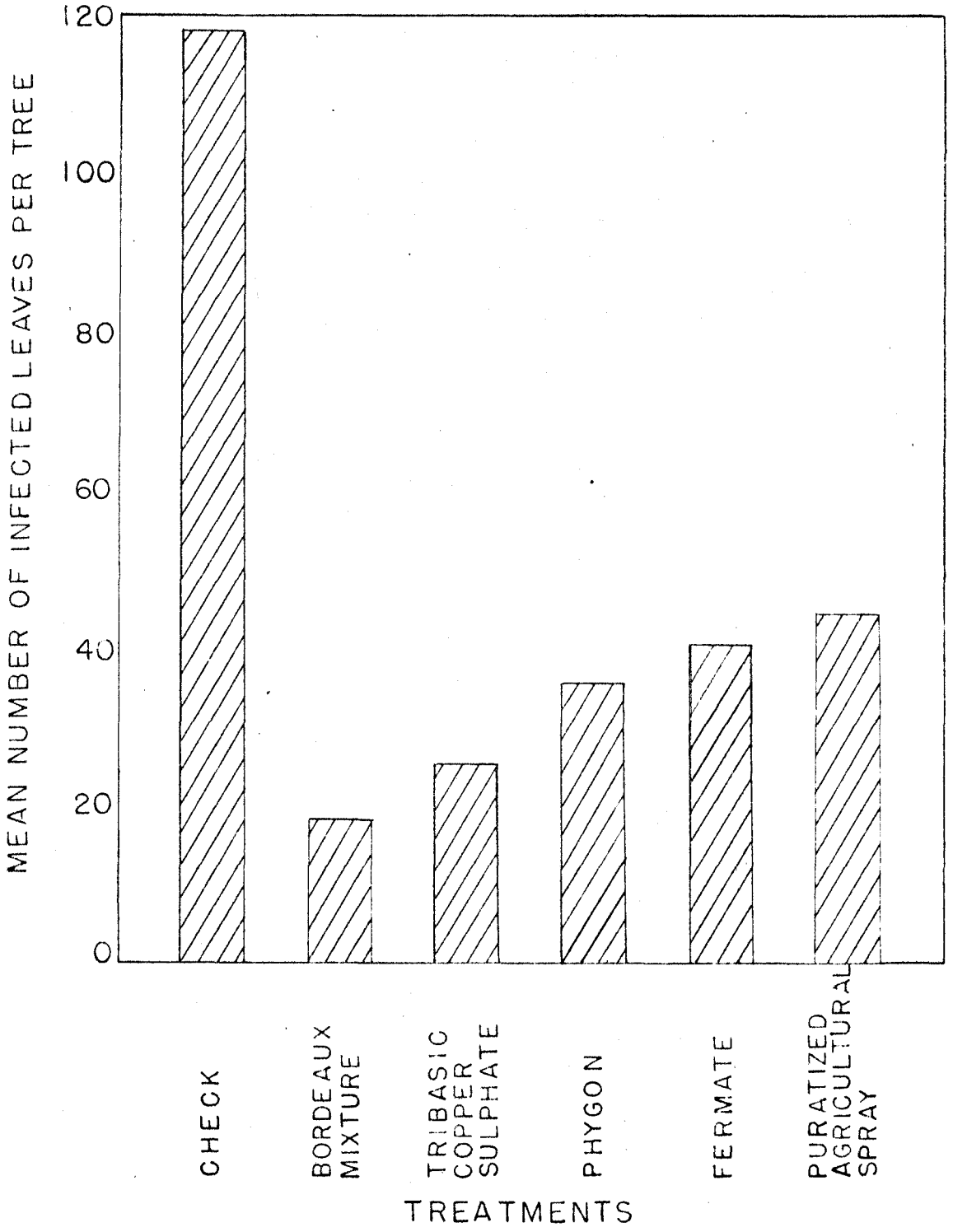

Fig. 16. Effectiveness of five fungicides in controlling sycamore anthracnose with four spray applications.

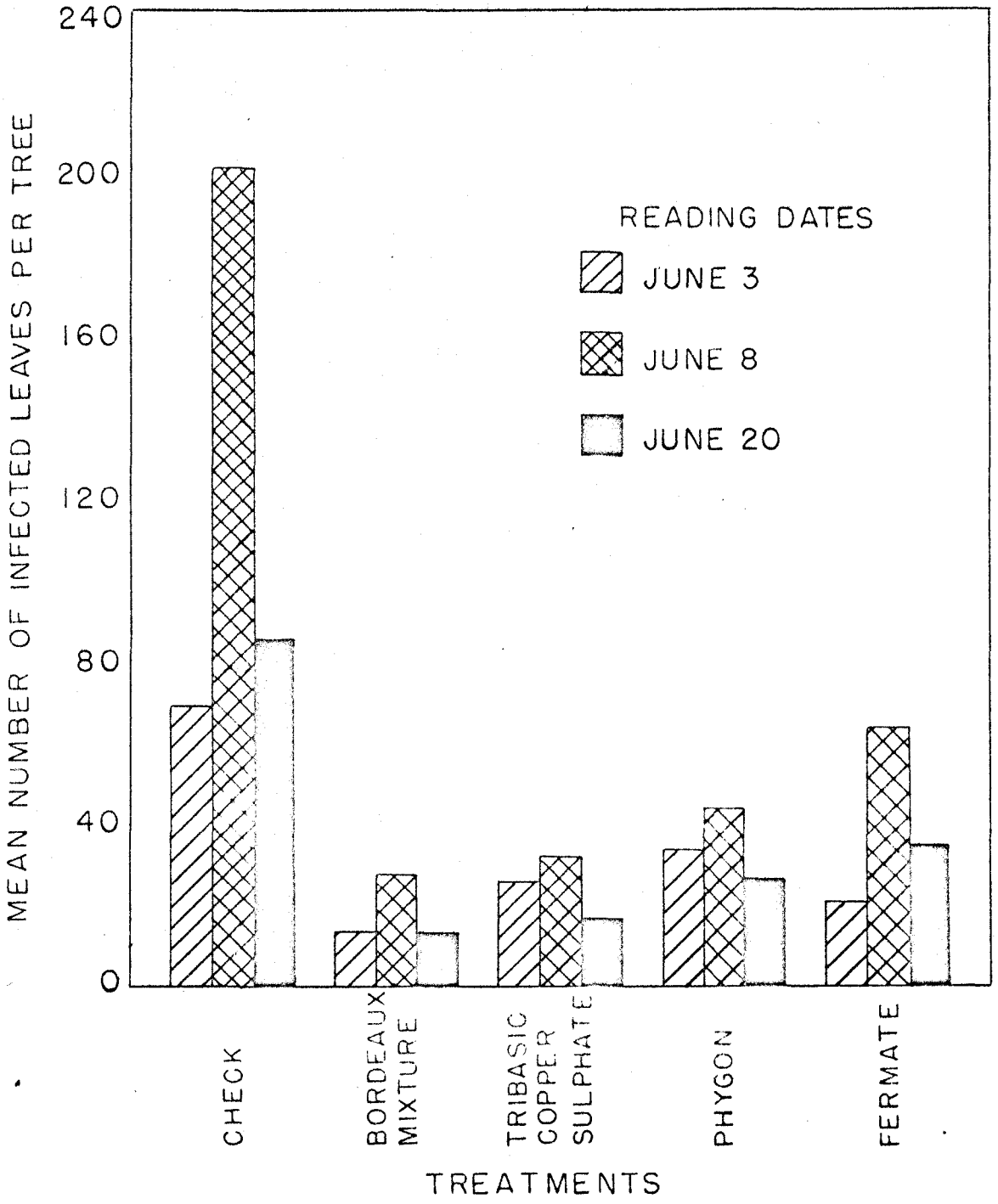

Fig. 17. Effectiveness of fungicides in controlling sycamore anthracnose as shown by the incidence of disease on three reading dates.

Table 24. Effectiveness of various spray date combinations in controlling sycamore anthracnose with phenylmercuri-triethanol ammonium lactate.

Spray date combinations**	No. leaves infected in replication on two June reading dates*								Mean infection per tree
	I		II		III		IV		
	7	30	7	30	7	30	7	30	
Check	102	125	562	35	274	55	134	67	85
Carry Over Check	121	81	575	84	188	39	86	29	75
Dormant Spray No. 1	149	31	182	15	120	63	435	34	64
Spray No. 2	193	43	220	40	350	59	275	41	76
Spray No. 1, 2	61	38	112	16	81	24	118	18	29
Spray No. 2, 4	288	101	357	63	196	38	301	50	87
Spray No. 2, 3	150	28	396	37	273	27	220	45	73
Spray No. 1, 2, 3	46	21	129	16	138	27	156	18	34
Spray No. 1, 3, 4	246	93	450	33	177	44	105	19	73

* On 2 central trees in each replication

**Spray No. 1 - dormant spray, applied April 7; Spray No. 2 - broken bud spray, applied May 13; Spray No. 3. - young leaf spray, applied May 27; Spray No. 4 - mature leaf spray, applied June 16.

Table 25. Analysis of variance for spray date combinations in controlling sycamore anthracnose with phenyl-mercuri-triethanol ammonium lactate.

Sources of variation	Degrees of freedom	Mean square	F
Treatments	8	6,873	1.86#
Replication	3	11,079	
Error	24	3,693	

Not significant

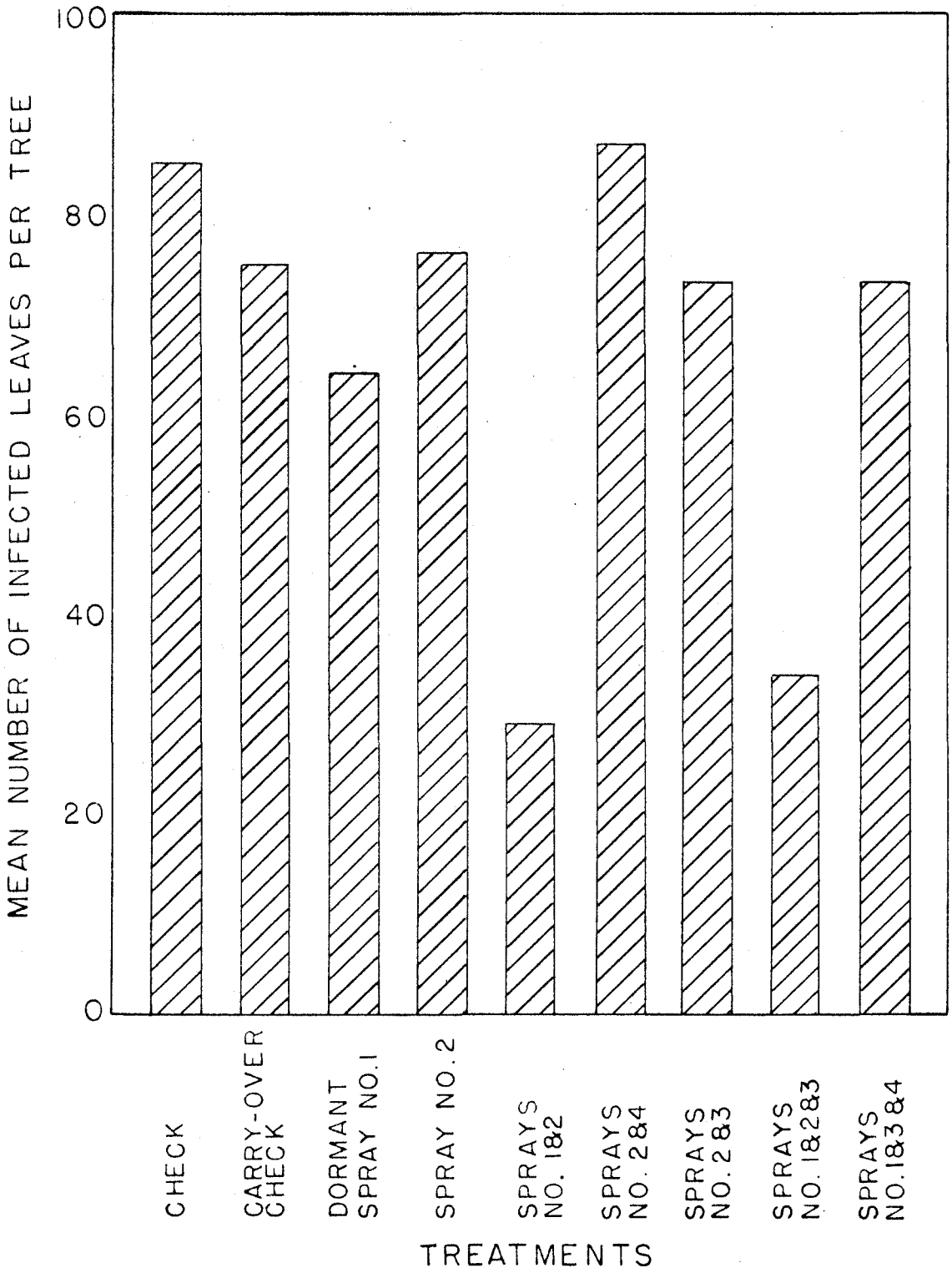

Fig. 18. Effectiveness of various spray date combinations in controlling sycamore anthracnose in 1950 with phenyl-mercuri-triethanol ammonium lactate.

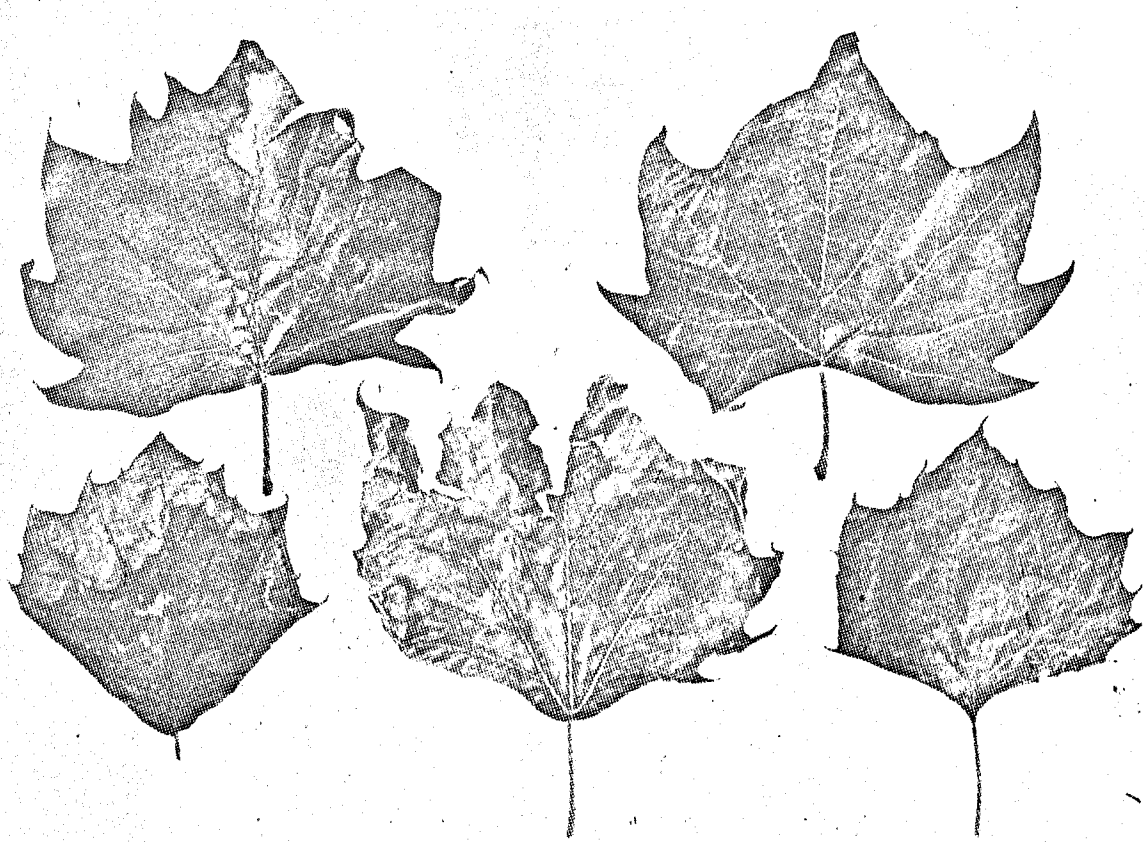

Fig. 19. Sycamore leaves in top row showing typical anthracnose infection along the veins; below, injury between the veins and along leaf margins caused by phenylmercuri-triethanol ammonium lactate.

DISCUSSION

The data obtained in these studies clarify the relationships of the sexual and conidial stages of anthracnose fungi found on oak and sycamore. The sexual stages produced on overwintered leaves from white oak and sycamore were similar in all respects except the perithecial necks were long on white oak tissue and short on sycamore. The conclusion that this difference is not sufficient reason for separation of these fungi is in agreement with Edgerton's (13) findings. It is possible that beak length is dependent on the substrate and may be influenced by nutrition or other factors. Attempts to produce perithecia on sterilized sycamore and oak leaves were not successful. However, what appeared to be perithecial initials were formed, and if adequate moisture had been supplied for long term incubation it is believed perithecia would have developed.

Conidial isolates from the sycamore group and conidia from single ascospores from white oak and sycamore have the same shape, size and cultural characteristics. Isolates from sycamore were able to infect both white oak and sycamore. The evidence indicates that all isolates of the sycamore group should be included in Gnomonia veneta.

The conidia of the oak group isolates are larger and more uniformly shaped than those of the sycamore group. Conidia of the oak group type were never obtained from single ascospore cultures. Although this group

is distinct, there were indications throughout the course of this study that the oak group is closely related and may be only a variant of Gnomonia veneta. For example, isolates of the oak group were repeatedly obtained in the spring from leaves of a white oak tree. Leaves from this same tree were overwintered and from them were obtained perithecia with long necks typical of the sycamore group from white oak. Ascospore cultures from these perithecia produced conidia also typical of the sycamore group. Hence from one tree isolates were obtained of both the oak and sycamore group. It is possible that both groups were present in this tree and simply were isolated at different times. However, it is also possible that mycelium which produces conidia typical of the oak group may produce perithecia from which isolates typical of the sycamore group can be obtained. If the latter possibility is true, then the oak group is a variant of the sycamore group.

Further evidence that a close relationship may exist between the oak and sycamore groups was obtained on one occasion when sectoring of isolates from both groups occurred on agar containing sodium oleate. Plates inoculated with an isolate from the sycamore group produced conidia in the sectored area which were of the oak group type. Plates inoculated with an isolate from the oak group produced conidia in the sectored area which were of the sycamore group type. Since these isolates had been reduced to single cells, there was no possibility of two organisms being present. Repeated attempts to duplicate these phenomena, however, were not successful.

Westerdijk and Van Luijk (63) found larger conidia on anthracnose infected oak leaves than on sycamore. Conidia from leaves of Quercus pedunculata, Q. ruba and Q. coccinia were 12.3, 13.2 and 12.6 μ in length, respectively. These measurements agree with those for conidia from Quercus alba, Q. borealis, Juglans nigra and Ulmus americana of the oak group which were 14.1, 12.6, 15.0 and 12.6 μ in length, respectively. It would appear the isolates they obtained from oak were similar to those designated as the oak group, and would explain their proposal that isolates from oak and sycamore be called separate species. Their measurements on sycamore conidial isolates were in agreement with those found in this study.

Although the above fragmentary evidence is to the contrary, the oak group must be considered distinct from Gnomonia veneta. It is suggested these isolates be called Gloeosporium quercinum West. until they can be identified with a sexual stage. This name was proposed by Westerdijk and Van Luijk for the isolates they obtained from oak. To further establish the identity of the oak group, leaves of white oak must be artificially infected with known isolates of the oak group and overwintered for perithecial formation.

It was shown for the first time that anthracnose fungi from oak and sycamore can be separated on the basis of growth factor requirements. Isolates of anthracnose fungi from white oak (oak group) have a complete deficiency for inositol, which heretofore has not been reported for parasitic fungi. An isolate from sycamore had previously been shown to require biotin and thiamin, and good growth of this isolate occurred when thiamin

alone was added to the basal medium (29). The conidial isolates from sycamore tested in this study, however, would not grow when only thiamin was added. This suggests that other strains of Gnomonia veneta may exist. Different strains may be developed when the sexual stage is formed on overwintered leaves. However, since ascospores are of minor importance in the disease cycle, it is more likely that the growth factors available to the fungus from the host tissue are responsible for the development of new strains. If growth factors are supplied in sufficient amounts for growth, the ability of the fungus to synthesize these materials may be lost. The possible separation of other strains of anthracnose fungi from oak and sycamore needs further study with many more isolates from both oak and sycamore, in addition to single ascospore isolates from these hosts to demonstrate this effect.

When fungi show a deficiency for two or more vitamins it becomes necessary to consider quantitatively the amount of growth in the presence of the vitamins singly and in combination. Conidial isolates from sycamore showed deficiencies for biotin, inositol and thiamin. When all supplements except biotin were added to the basal medium, 9.25 mg. of growth were recorded, for all supplements except thiamin, 18.5 mg., and for all supplements except inositol, 43 mg. When biotin and thiamin were added together good growth occurred, but when inositol was added to this combination, growth was decreased. This decrease in growth cannot be explained on the basis of these tests, but by varying the concentrations of the essential vitamins and mineral salts an explanation may be obtained.

No immediate explanation is available as to why oleic acid and linoleic acid stimulate spore germination and respiration. These materials may serve as essential growth factors in the same fashion as citrates and sucrose stimulate spore germination for many other fungi. It is more likely, however, that they facilitate wetting of the spores or change the permeability of the spore membranes. It is possible that the increase in germination obtained when droplets of spore suspension were dried slowly, may also be associated with changes in spore membranes. Spore germination stimulation cannot be explained on the basis of hydrogen-ion concentration since stimulation occurred for some materials at pH 7.5, and the optimum hydrogen-ion concentration for germination in a citric acid-phosphate buffer was pH 4 to 5.

Some of the isolates of the oak group were obtained from two- and three-year old wood of red oak, white oak and elm. Gnomonia veneta was recovered from the midrib of a seemingly healthy sycamore leaf. These fungi apparently have a tendency to become systemic in branches, twigs and petioles and may or may not induce symptoms. For example, it was shown Gnomonia veneta can grow from infected sycamore leaves through petioles and cause twig blight or from infected twigs through petioles and cause leaf blight. This invasion of woody tissue is local, for although twigs are killed and cankers are formed on larger branches, the fungus is restricted to these areas.

SUMMARY

Anthracnose fungi isolated from infected oak and sycamore were divided into two rather well defined groups. The sycamore group included conidial isolates from sycamore (Platanus occidentalis L.), white oak (Quercus alba L.) and bur oak (Quercus macrocarpa Michx.) in addition to the sexual stages produced on overwintered leaves of white oak and sycamore. The conidia from ascospores and naturally infected tissue were indistinguishable, regardless of source. The ascogenous stage from white oak and sycamore differed little, except for perithecial beak length. Conidial isolates from sycamore were capable of infecting white oak and sycamore. The evidence indicated all these isolates are included in Gnomonia veneta (Speg. & Sacc.) Klebahn.

The oak group included conidial isolates from red oak (Quercus borealis Michx.), white oak, American elm (Ulmus americana L.) and black walnut (Juglans nigra L.). The conidia from this group were larger than those from the sycamore group and were more uniformly shaped. The cultural characteristics of the two groups were different. Conidia of the oak group were never obtained from single ascospore cultures. Conidial isolates from white oak (oak group) were capable of infecting both white oak and sycamore. Although there was some evidence the oak group was closely related to Gnomonia veneta, it was proposed these isolates be referred to as

Gloeosporium quercinum West. until proof of their identity can be more definitely established.

Isolates from both groups grew on natural media such as potato-dextrose or yeast agar, but did not grow on such synthetic media as Czapek's or Elliott's agar. A synthetic medium fortified with certain vitamins was found to support growth.

All isolates of the oak group tested gave the same response by exhibiting a complete deficiency for inositol, and a partial deficiency for thiamin. When a representative isolate of this group was supplied with the necessary vitamins and an organic source of nitrogen, such as vitamin-free casein hydrolysate, more growth occurred than when only inorganic nitrogen was present in the basal medium. The rate of growth was more than doubled when these isolates were aerated by shake culture. Isolate B-3-3 of the oak group was sensitive to 0.1 ppm. of inositol.

Within isolates of the sycamore group two strains were distinguished by their growth factor requirements: (1) Isolates from sycamore showed nearly a complete deficiency for biotin and partial deficiences for thiamin and inositol, however, there was some evidence inositol can partially replace biotin. When biotin and thiamin were added to the basal medium good growth occurred. That these isolates grew when biotin alone was added to the basal medium distinguished them from the oak group where growth occurred only when inositol was added. (2) Isolates from white oak (sycamore group) exhibited a different response. They grew without the addition of vitamins to the basal medium, and grew better, but about equally well on inositol and thiamin, or biotin and thiamin.

Thiamin was found to increase the sporulation of all isolates tested. Six times as much sporulation was recorded when thiamin, biotin and inositol were added to the basal medium as was recorded when all supplements, except thiamin, were added.

Oleic and linoleic acid emulsions, and sodium oleate solutions (adjusted to pH 7.5) stimulated the germination of spores from isolates of both the sycamore and the oak groups. These materials also sharply increased the respiratory rate of a representative isolate of the oak group.

The optimum hydrogen-ion concentration for germination of spores from a representative isolate of the oak group was pH 4 to 5.

When droplets of spore suspensions, from an isolate of the oak group, were incubated for 24 hours on glass slides in relative humidities from 100 to 95 percent, maximum germination occurred at 97 percent.

One-year-old white oak seedlings were successfully inoculated with isolates from the oak and sycamore groups by using spore germination stimulants and a gradual reduction of the relative humidity in the moist chambers.

It was shown for the first time that Gnomonia veneta was able to grow from infected leaves to healthy twigs and cause twig blight. Also, it was shown that the fungus is able to grow from infected twigs into healthy leaves and cause leaf blight.

In a replicated test on sycamores in 1950, Gnomonia veneta was effectively controlled by four spray applications. A significant difference was found between the checks and the treatments, but differences between

treatments were not significant. However, on the basis of two years' results Bordeaux mixture appeared to be the best material for controlling sycamore anthracnose. In a test with phenylmercuri-triethanol ammonium lactate on large sycamore trees, the combination of a dormant spray and a broken bud spray was most effective in controlling the disease. This chemical caused some injury to leaf surfaces.

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ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Dr. W. H. Bragonier for council and guidance and invaluable assistance in the preparation of the manuscript. Acknowledgment is also given to Dr. J. C. Gilman for many suggestions and helpful advice during the course of this study.

Much of the field work was accomplished through the cooperation afforded by members of the State Conservation Commission, especially Mr. W. A. Rush. Appreciation is extended to the Des Moines City Parks Department and to Mr. Fred Mansfield for cooperation in carrying out the spray experiments.